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ON PROTEOLYTIC ENZYMES. I.

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NUMEROUS investigators have studied the proteolytic enzymes in plants and their work appears to have been carried on from two different points of view. The physiological chemists working with vegetable proteases have made careful studies of the products and nature of the action of enzymes of marked activitity. Of such work, the investigations carried on by CHITTENDEN and his pupils on bromelin are excellent examples. In studies of this kind the enzyme is the important thing, the rôle of the enzyme in the physiology of the plant is secondary. On the other hand, the studies of plant physiologists have been directed less to working out the nature of the enzymes and more to locating them and to discovering their function in the life of the plant. Of such researches the intensive studies of BUTKEWITSCH on germinating seeds, and the extensive ones of VINES on all sorts of plant tissues, are good illustrations.

Looking at the subject from the point of view of plant physiology, we may conceive of proteolytic enzymes as being digestive, *i. e.*, acting on stores of proteid, as in germinating seeds; or metabolic, *i. e.*, acting on the proteids in the life of the vegetable cells. The idea of enzymes digesting the reserve proteids was the first to occur to physiologists. Acting on the hypothesis of the existence of such enzymes, numerous investigators have examined germinating seeds, using a variety of methods, and obtaining results which have not always been in harmony with the hypothesis or with each other.

The view that wherever we find proteids in an organism, there we should always find a proteolytic enzyme, is not a new one; the experimental facts to support such a conception have been slow

in coming. Manifestly the first step in proving this assumption is to show that proteolytic enzymes are present in all parts of all living plants, or to demonstrate their presence in a sufficient number of places to warrant the conclusion that they are universally present. Such extensive researches have not been undertaken until the last few years.

I shall briefly review the results of the various investigations, taking first the studies on the digestive enzymes, as I have called them, and then those on the metabolic enzymes.

GORUP-BESANEZ[1] appears to have been the first to examine seeds for proteolytic enzymes. Using the Wittich glycerin method he obtained protease preparations from the resting seeds of hemp, flax, and vetch, and from the germinating barley; he failed to find enzymes in ungerminated barley, lupin seeds, *Secale cornutum*, pine, and corn, and in seedlings of almonds and beans.

VAN DER HARST[2] found that a glycerin extract of the cotyledons of germinating beans would digest fibrin.

The first careful study of a seed proteolytic enzyme was that of J. REYNOLDS GREEN[3] who investigated the germinating seeds of *Lupinus hirsutus*. GREEN'S results showed conclusively that these plants contain an active enzyme, capable of attacking fibrin, as well as the proteids of the seeds themselves. The enzyme was not present in the resting seeds. Later GREEN discovered a similar enzyme in the seedlings of the castor oil bean.[4]

NEUMEISTER[5] undertook the study of a number of seeds and seedlings, using a method different from that of any previous investigator in this field. Pieces of fibrin were soaked in the watery extracts from the tissues to be tested; subsequently the fibrin was transferred to a weakly acid solution and allowed to digest. In case a proteolytic enzyme was present it was taken up by the fibrin, and in the acid medium proteolysis of the fibrin took place. The value

[1] GORUP-BESANEZ, Ber. Deutsch. Chem. Gesells. **7**:1478. 1874; **8**:1510. 1875; and **9**:673. 1876.

[2] VAN DER HARST, Moonblad voor Naturwetensch. 1867, Sept. 20.

[3] GREEN, J. REYNOLDS, Phil. Trans. **178**:(B) 39. 1887.

[4] GREEN, J. REYNOLDS, Proc. Roy. Soc. **48**:370. 1890.

[5] NEUMEISTER, Zeitschr. für Biologie **30**:447. 1894.

of the negative results by this method has been called in question by FRANKFURT.[6] NEUMEISTER's results may be summarized as follows:

POSITIVE RESULTS	NEGATIVE RESULTS
Barley seedlings—sprouts 3 cm long.	Barley seeds.
Barley seedlings—sprouts 5 cm long.	Barley seedlings—sprouts 0.5 to 1 cm.
Barley seedlings—sprouts 15–20 cm long.	
Poppy seedlings.	Poppy seeds.
Beet seedlings.	Beet seeds.
Corn seedlings—sprouts 10 cm long.	Corn seeds.
Corn seedlings—sprouts 16 cm long.	Lupin seeds.
Wheat seedlings—sprouts 17 cm long.	Lupin seedlings—sprouts 3 cm.
	Lupin seedlings—sprouts 12 cm.
	Lupin seedlings—sprouts 20 cm.
	Vicia seeds.
	Vicia seedlings.
	Pea seeds.
	Pea seedlings.
	Rye seeds.
	Rye seedlings.
	Oat seeds.
	Oat seedlings.

A number of different investigators have worked on malt; the evidence shows that the germinating barley contains a proteolytic enzyme. WEISS[7] thinks there are two, a peptic and a tryptic enzyme, a view which is supported by FERNBACH and HUBERT,[8] but questioned by VINES.

In the course of the extensive studies of FERMI and BUSCALIONI[9] on proteases in plants, they tested a number of seeds and found that the ripe seeds of several plants contain an enzyme capable of liquefying gelatin. They likewise found that the germinating seeds of some plants contained such enzymes, but in other cases no evidence of their presence could be found.

BUTKEWITSCH[10] made a careful study of the germinating seeds of two lupins, *L. angustifolius* and *L. luteus*, and a somewhat shorter

[6] FRANKFURT, Landw. Versuchsstat. **47**:466. 1896.

[7] WEISS, Zeitschr. für Physiol. Chem. **31**:79.

[8] FERNBACH and HUBERT, Compt. Rend. **130**:1783.

[9] FERMI and BUSCALIONI, Centralbl. Bact., etc. II. **5**:24. 1899.

[10] BUTKEWITSCH, Zeitschr. Physiol.-Chem. **32**:1. 1901.

examination of *Ricinus major* and *Vicia Faba*. His experiments show that the proteids in the sprouting seeds of all these species will undergo autolysis, giving rise to products not precipitable by phosphotungstic acid. From *L. luteus* he made a proteid preparation by the Wittich method (solution in glycerin and precipitation by alcohol) which acted on "conglutin" with the formation of leucin and tyrosin, but not of asparagin.

VINES[11] found some difficulty in getting a formation of tryptophan in the autolysis of germinating seeds of *Vicia Faba;* the reaction was readily obtained, however, if Witte peptone was added to the mixture and digestion allowed to proceed. He found that the embryo of the wheat contains an enzyme which readily attacks Witte peptone and apparently has some slight action on some of the proteids of the embryo.

As stated above, the first step in demonstrating that proteases are universally at work where proteids occur, is to show that all living plant tissues contain proteolytic enzymes. Investigations of this sort have been undertaken but recently. The older researches, which did not have this purpose, did tend to show that active proteases occur in places other than germinating seeds. The papaïn of the pawpaw fruit, the bromelin of the pineapple juice, the cradeïn of the latex and juice of the fruit of the fig, and the protease of the Kachree gourd and of *Anagallis arvensis* have been known for some time, but these plants were looked upon as exceptional.

FERMI and BUSCALIONI[12] were the first to make a systematic examination of a large number of plant tissues. Their method consisted in placing the objects to be tested on gelatin containing phenol as an antiseptic. If the gelatin was liquefied by the tissue or tissue extract, a protease was judged to be present. The positive results of this method are valuable; negative results cannot be taken as conclusive evidence that no proteolytic enzyme is present, since all proteases do not attack gelatin, and phenol is said to have an inhibitory influence on the action of some enzymes. These investigators succeeded in showing that proteases occur in a great diversity of plants and of plant parts. Their negative results are sufficiently numerous

[11] VINES, Annals of Botany **16**: 12. 1902.

[12] FERMI and BUSCALIONI, Centralbl. Bact., etc., II. **5**: 24. 1899.

to cause some doubt in respect to the universal occurrence of proteases in living tissues, and the positive results were obtained with such a diversity of tissues that they do not lead to any definite hypothesis of the physiological rôle of these enzymes. In view of the evidence of VINES' researches, it appears probable that a more thorough study of the tissues examined by FERMI and BUSCALIONI would show that proteases are present there which cannot act under the conditions of their experiments. Their positive results support the theory of the universal distribution of proteolytic enzymes, their negative results do not disprove it.

Finally we come to a consideration of the results of VINES.[13] He believes that all vegetable proteases are proteolytic in distinction from peptonizing, *i. e.*, that they change the proteids which they attack into their ultimate cleavage products, amido-acids, hexon bases, etc., and do not stop, like pepsin, with the albumoses and peptones in preponderance. Acting on this well-justified assumption, he expects to find tryptophan as the result of the action of vegetable proteases, and uses as a test for proteolysis the progressive formation of tryptophan as shown by the color reaction with chlorine or bromine water. He uses the method of autolysis, allowing the enzymes of a tissue to digest the proteids of that tissue and from time to time tests samples of the mixture for tryptophan. He frequently adds other proteids to the digestion, especially Witte peptone. The only objection to the method is that some proteids do not contain the tryptophan group, or only a small amount of it. A negative result by VINES' method with a tissue containing such a proteid would not mean much. This probably explains the differences between the results obtained by VINES and BUTKEWITSCH in their examinations of the germinating seeds of *Vicia Faba*, since OSBORNE and HARRIS[14] have shown that the proteids of this seed give but a faint Adamkiewicz reaction, a reaction which HOPKINS and COLE[15] have shown to be due to the presence of the tryptophan group in the proteid molecule. By using Witte peptone, VINES obtains evidence of proteolysis in cases where the tissue contains

[13] VINES, Annals of Botany **16**:1. 1902; **17**:237 and 597. 1903; and **18**:289. 1904.

[14] OSBORNE and HARRIS, Jour. Am. Chem. Soc. **25**:853. 1903.

[15] HOPKINS and COLE, Jour. Physiology **27**:419. 1901.

no peptonizing enzyme, but does have an enzyme which, like erepsin of the animal body, attacks albumoses and peptones. In cases where all the trials were made with the addition of Witte peptone, it is impossible to say whether there is an enzyme present of the trypsin type (tryptase), the erepsin type (ereptase), or both. In cases where a positive result was obtained both with or without the addition of Witte peptone, a tryptase is present if, as is probable, the tissue contains no marked quantity of albumoses or peptones; an ereptase may also be present and in the table its presence is indicated as questionable. When no tryptophan reaction is given on autolysis, except after the addition of a proteose, I have assumed that no tryptase was present, an assumption which, as pointed out above, is not entirely justified because certain proteids give no reaction for the tryptophan radical (see table).

This table records but three failures to obtain evidence of a protease, the pulp of the apple, the juice of the orange, and the sap of the bleeding birch tree (*Betula alba*), which last is not a cell extract. The two failures, the apple and the orange juice, are scarcely sufficient to prevent the acceptance of these results as evidence of the universal occurrence of proteases in living plant tissues.

No mention has been made of the proteolytic enzymes of the insectivorous plants and the bacteria and lower fungi. Some of the latter are doubtless endo-enzymes and belong to the group which I have termed metabolic enzymes. The others are apparently secretions designed to render food materials available and should be classed with the digestive enzymes.

The results of VINES tend to show that there are enzymes of the erepsin type widely distributed in plants, yet the evidence is not wholly conclusive and he has made no attempt to isolate such enzymes. DELEZENNE and MOUTON[16] made extracts from several fleshy fungi, *Amanita muscaria*, *Amanita citrina*, *Hypholoma fasciculare*, and *Psalliota* (*Agaricus*) *campestris*, which were judged to contain an ereptase since they digested proteoses but not fibrin. VINES finds, however, that *Agaricus campestris*, which DELEZENNE and MOUTON found to yield an especially active ereptase, is capable of autolysis and will digest fibrin if the fibrin is not boiled. The unboiled fibrin

[16] DELEZENNE and MOUTON, Compt. Rend., April 9, 1903.

Tissue	Tryptase	Erepsin	Erepsin or Tryptase or both
Milk of *Cocos nucifera*	−	+	..
Pilei of *Agaricus campestris*	+	+ ?	..
Unripe seeds of *Pisum sativum*	+	+ ?	..
Fruit of *Cucumis melo*	+	+ ?	..
Fruit of *Cucumis sativus*	+	+ ?	..
Fruit of *Cucurbita pepo-ovifera*	..	..	+
Fruit of *Ecballium Elaterium*	−	+	..
Fruit of *Musa sapientum*	+	+ ?	..
Fruit of *Lycopericum esculentum*	+	+ ?	..
Fruit of *Pyrus malus* (pulp)	−	−	−
Fruit of *Pyrus malus* (skin)	−	+	..
Fruit of *Pyrus communis*	−	+	..
Fruit of *Citrus aurantium* (juice)	−	−	−
Fruit of *Citrus aurantium* (peel)	?	+	..
Fruit of *Vitis vinifera*	..	..	+
Sap of *Betula alba*	−	−	−
Shoots of *Crambe maritima*	− ?	+	..
Yeast	+	+ ?	..
Latex of *Euphorbia Characias*	−	+	..
Latex of *Lactuca sativa*	+ ?	+	..
Stems of *Dahlia variabilis*	−	+	..
Stems of *Cucurbita pepo-ovifera*	+	+ ?	..
Stems of *Mirabilis Jalapa*	+	+ ?	..
Stems of *Helianthus tuberosus*	+	+ ?	..
Stems of *Cuscuta*	−	+	..
Leaves of *Spinacia oleracea*	+	+ ?	..
Leaves of *Dahlia variabilis*	+	+ ?	..
Leaves of *Tropaeolum majus*	+	+ ?	..
Leaves of six other species of plants	+	+ ?	..
Leaves of *Holcus mollis* and three other species of plants	..	..	+
Bulbs of *Tulipa* and two other plants	+	+ ?	..
Tubers of *Solanum tuberosum*	+	.....	.....
Tubers of *Helianthus tuberosus*	+	+ ?	..
Roots of *Dahlia variabilis*	+	+ ?	..
Roots of *Brassica Rapa* and seven other plants	−	+	..

which VINES used would not digest itself and he believes its solution was not due to any enzyme which the fibrin itself contained. VINES agrees with DELEZENNE and MOUTON in believing that this fungus contains an enzyme of the erepsin type.

EXPERIMENTAL DATA

In undertaking the study of the proteolytic enzymes I have had two ends in view. In the first place it was thought desirable to test a number of different plant tissues in order to confirm, if possible, the results of VINES. The second and more important part of the investigation was to make a careful study of the enzymes in some

one or two plants, tracing their occurrence and studying their nature throughout the life history of the plant. In this way it was hoped that some results might be obtained which would throw light on the nature and functions of the proteases in the life of plants. For this study the common field bean, *Phaseolus vulgaris*, which has a large proteid content in its seeds and shows marked vigor and rapidity in its growth, was selected. The work on the enzymes of Phaseolus has proved so absorbing that scant attention has been paid to the testing of various plants. The few positive results which have been obtained will be set down at this time.

SPINACIA OLERACEA.—Fresh leaves of spinach were carefully picked over to remove all imperfect tissue and then ground up and the juice removed by a screw press. The residue was rubbed up with a little water and the fluid again pressed out. The turbid dark green extract was examined as follows:

Flask no. 1—50^{cc} extract—boiled.
Flask no. 2—50^{cc} extract—unboiled.
Flask no. 3—50^{cc} extract + .5 ^{gm} Witte peptone—boiled.
Flask no. 4—50^{cc} extract + .5 ^{gm} Witte peptone—unboiled.
Flask no. 5—50^{cc} extract + .5 ^{gm} Witte peptone + 0.25 ^{gm} citric acid—boiled.
Flask no. 6—50^{cc} extract + .5 ^{gm} Witte peptone + 0.25 ^{gm} citric acid—unboiled.

The antiseptic was chloroform, the length of digestion 30 hours. Ten cubic centimeters of each digestion were tested by acidifying with acetic acid, drying, taking up in alcohol and filtering off the residue, drying the alcoholic extract, taking up the residue in water, and applying the tryptophan test with chlorine water. The results were negative except in the case of no. 4, which showed a fair tryptophan reaction. This result shows that the leaves of spinach contain an enzyme capable of attacking Witte peptone, but not acting on the proteids of the tissue, or if it does these proteids do not yield tryptophan on hydrolysis.

BRASSICA OLERACEA.—The leaves of a half-matured cabbage, some of which were green and some etiolated, were run through a chopping machine and the pulp macerated with water. The fluid was removed by a screw press and the pale green turbid extract filtered. This extract was faintly acid to litmus, but not to lacmoid, and yielded a coagulum on boiling, the filtrate from

which gave a faint tryptophan reaction. The extract was examined in the following way:

Flask no. 1—50^{cc} extract.
Flask no. 2—50^{cc} extract—boiled.
Flask no. 3—50^{cc} extract + 1^{cc} 4 per cent. HCN
Flask no. 4—50^{cc} extract + 1^{cc} 4 per cent. HCN—boiled.
Flask no. 5—50^{cc} extract + 1^{cc} 4 per cent. HCN + 0.5 ^{gm} Witte peptone.
Flask no. 6—50^{cc} extract + 1^{cc} 4 per cent. HCN + 0.5 ^{gm} Witte peptone—boiled.
Flask no. 7—50^{cc} extract + 0.25 Na_2CO_3.
Flask no. 8—50^{cc} extract + 0.25 Na_2CO_3—boiled.

Ten drops of toluol were added to nos. 1, 2, 7, and 8. The flasks were corked and placed in the incubator. After twenty hours' digestion 10^{cc} was removed from each flask, boiled, filtered, and 1^{cc} of acetic acid added to each filtrate. The tryptophan test with chlorine water showed that proteolysis had taken place in all the unboiled digestions except no. 3; that in no. 1 was feeble compared with the action in nos. 5 and 7. These results show that although the enzymes of the cabbage leaves act on the proteids of the tissue, yet that action is slight compared with that exerted upon Witte peptone. The tissue residue from which the extract had been obtained was shaken up with water and toluol, a small portion removed and boiled, and Witte peptone added to both parts. After two days' digestion a sample of the fluid in each flask was tested for tryptophan; the unboiled digestion gave a strong reaction, the boiled one a very faint reaction. The fluid was removed as far as possible from the unboiled digestion mixture, concentrated to a syrup, treated with several volumes of alcohol to remove the proteoses and peptones, and the filtrate concentrated to a thick syrup. After standing, very characteristic crystals of both leucin and tyrosin separated.

DAUCUS CAROTA.—Blossoms of the wild carrot were ground in a mortar with water. The mashed tissue and water were divided into four parts and placed in test tubes.

Tube no. 1—tissue + H_2O + toluol + uncoagulated egg albumen.
Tube no. 2—tissue + H_2O + toluol + uncoagulated egg albumen—boiled.
Tube no. 3—tissue + H_2O + Witte peptone + toluol.
Tube no. 4—tissue + H_2O + Witte peptone + toluol—boiled.

The egg albumen was added because a test of the tissues with

Adamkiewicz reaction, using glyoxylic acid and concentrated sulphuric acid, gave no well-defined positive result. After two days, digestion no. 3 was found to give a weak tryptophan reaction, whereas none of the others gave a positive result. An experiment carried out in the same way with the developing seeds gave the same result except that the unboiled test with Witte peptone gave a much stronger reaction for tryptophan, showing that the proteolysis had been more vigorous. The evidence indicates that the enzyme present is an ereptase.

CASTANEA SATIVA AMERICANA.—The leaves of this tree were ground with sand in a mortar, the pulp mixed with water, divided between four test tubes, and examined as follows:

No. 1—toluol + uncoagulated egg albumen.
No. 2—toluol + uncoagulated egg albumen—boiled
No. 3—toluol + Witte peptone
No. 4—toluol + Witte peptone—boiled.

After two days in the incubator no. 3 gave a faint reaction for tryptophan, the others none. The original pulp from the leaves gave no Adamkiewicz reaction. The unripe seeds of the chestnut were ground to a paste and mixed with water. No Adamkiewicz reaction was given by this mixture, which was divided between four test tubes and examined in precisely the same way as the leaves. The result was qualitatively the same, but the tryptophan reaction in the unboiled trial with Witte peptone was very strong, showing the presence of an active protease, probably an ereptase.

PHASEOLUS MUNGO.—Three weeks old etiolated seedlings of this bean were rubbed up in a mortar and covered with glycerin and a little toluol. After extracting for twenty-two hours, the glycerin was somewhat diluted, strained through gauze, and filtered through absorbent cotton. This extract was tested as follows:

No. 1—10^{cc} extract + fibrin.
No. 2—10^{cc} extract boiled + fibrin.
No. 3—10^{cc} extract + Witte peptone.
No. 4—10^{cc} extract + Witte peptone—boiled.

Examined after three days' digestion no. 1 showed no digestion of the fibrin and gave no test for tryptophan, no. 3 gave a marked tryptophan reaction, and no. 4 none.

CUCURBITA MAXIMA.—The seeds of this plant and the young seedlings with radicles an inch and a half long were found to contain an ereptase of considerable power as shown by the action of their tissues on Witte peptone Under conditions for autolysis none seemed to take place, since no tryptophan reaction was obtainable, notwithstanding the fact that the proteids of this seed give a strong Adamkiewicz reaction. When the seedlings were thirteen days old, however, they were found capable of autolysis with the formation of tryptophan. The results show that the seeds contain an ereptase and that after germination has proceeded for some time a tryptase also makes its appearance.

CUCURBITA PEPO.—The seeds of the pumpkin give the same evidence of the presence of an ereptase as do those of the Hubbard squash. Two attempts were made to obtain preparations of this enzyme. A quantity of ground seeds of the pumpkin were whipped up in water, and the shells, which floated on the surface, were skimmed off. After extracting for several hours, the mixture was strained through gauze and an attempt made to filter it clear. The fluid appeared to have a quantity of emulsified fat in it, and even the repeated filtration through pulp filters failed to make any impression on it. The mixture was allowed to stand two days with toluol to prevent bacterial action and was then readily filtered, yielding a perfectly clear filtrate, which however possessed no proteolytic power. In the second attempt 45^{gm} of ground seeds were extracted with sufficient water to make 275^{cc} of milky looking extract which was found to have a marked action on Witte peptone. As soon as this extract was strained from the ground seeds it was mixed with an equal volume of saturated ammonium sulphate solution and allowed to stand over night. The precipitate was filtered off the next day, and when tested with Witte peptone was found to be very active, showing that the enzyme is either precipitated by half saturation of its solution with ammonium sulphate, or else it sticks to the proteid precipitated by the salt. The precipitate was whipped up in water, transferred to a parchment paper bag, and dialyzed for two days in running water. When taken from the dialyzer and filtered clear, the solution was found to be inactive on Witte peptone. These results show that this enzyme is very sensitive and that some

other means must be resorted to in order to obtain preparations of it free from the bulk of proteids, etc., in the tissue extracts.

STUDY OF THE PROTEASES OF PHASEOLUS VULGARIS

The beans used in the experiments to be described were of two sorts, the red kidney bean and the white medium field bean; there has been nothing to suggest that the proteases in these varieties are different, and in the records of experiments no mention will be made of which kind was used.

Resting seeds.—The resting seeds were tested a number of times for proteases; the results always showed that the seeds contained an enzyme capable of hydrolyzing Witte peptone with the formation of tryptophan, but incapable of digesting the phaseolin of the seed. Later in this paper will be found a more detailed description of this ereptase. The record of a typical experiment is given here.

Seeds of *Phaseolus vulgaris* which were known to germinate readily were ground to a fine meal, one gram of this powder placed in each of four test tubes, and further additions made as follows:

No. 1—15^{cc} H_2O + toluol.
No. 2—15^{cc} H_2O + toluol—boiled.
No. 3—15^{cc} H_2O + toluol + 0.3^{gm} Witte peptone.
No. 4—15^{cc} H_2O + toluol + 0.3^{gm} Witte peptone—boiled.

All were corked, shaken, and kept in the incubator at 37–39° C. for forty-one hours. At the expiration of that time the contents of each tube were boiled, acidified with acetic acid, and filtered. The filtrates were then tested.

No. 1—no biuret, Millon's, nor tryptophan reactions.
No. 2—not tested.
No. 3—strong tryptophan reaction.
No. 4—no tryptophan reaction.

Seeds soaked in water for twenty-four hours.—Seeds from the same lot used in the above experiment were soaked in distilled water for twenty-four hours, the hulls removed, and the cotyledons ground in a mortar and 1.5^{gm} of the paste tested in the same way as the dry bean meal, and with the same results.

Seeds soaked for forty-five hours.—The beans not used in the last experiment were soaked twenty-one hours longer, peeled, and the tests repeated, with the same results.

Seeds soaked twenty-four hours and germinated in sand three days.—At this stage the hypocotyls were about one inch long. The cotyledons were removed, ground in a mortar, and the paste tested as in the previous experiments. The result was the same, the Witte peptone was attacked and the seed proteids were not.

Seeds soaked twenty-four hours and germinated six days.—The cotyledons from the young plants with hypocotyls two to three inches long were removed and ground to a paste. One gram of this was placed in each of six test tubes and other additions made as follows:

No. 1—10^{cc} H_2O + toluol.
No. 2—10^{cc} H_2O + toluol—boiled.
No. 3—10^{cc} H_2O + 0.1 per cent. of citric acid + toluol.
No. 4—10^{cc} H_2O + 0.1 per cent. of Na_2CO_3 + toluol.
No. 5—10^{cc} H_2O + 0.2^{gm} Witte peptone + toluol.
No. 6—10^{cc} H_2O + 0.2^{gm} Witte peptone—boiled + toluol.

After nineteen hours in the incubator the contents of each tube were boiled, acidified, filtered, and the filtrates tested with these results:

No. 1—no tryptophan nor biuret reactions, Millon's?
No. 2—not tested.
No. 3—no tryptophan reaction, biuret? Millon's?
No. 4—no tryptophan reaction, biuret? Millon's?
No. 5—strong tryptophan reaction.
No. 6—no tryptophan reaction.

Seeds soaked twenty-four hours and germinated ten days in the dark.—The cotyledons at this age of the plant were comminuted and tested in tubes as follows:

No. 1—ground cotyledons + H_2O + toluol.
No. 2—ground cotyledons + H_2O + toluol + uncoagulated egg albumen.
No. 3—ground cotyledons + H_2O + toluol + edestin.
No. 4—ground cotyledons + H_2O + toluol + Witte peptone.

After eighteen hours in the incubator the tests showed that only the Witte peptone had undergone proteolysis.

Cotyledons of eleven-day old seedlings grown in light.—Beans allowed to germinate in washed sand, with a good supply of light, for eleven days had attained a height of five to six inches and had two well developed leaves besides the shriveled cotyledons. The cotyledons were found still to contain an active ereptase.

Cotyledons of etiolated seedlings thirteen days old.—These cotyledons gave convincing evidence of the presence of ereptase, but no tryptase.

Besides testing the cotyledons of germinating beans by the method of autodigestion, extracts were also prepared with glycerin and 20 per cent. alcohol. The tests applied to these extracts showed that they were incapable of digesting the bean proteids or boiled fibrin.

It should be noted that OSBORNE and HARRIS[17] have found that phaseolin, the globulin which makes up the main proteid store of the resting bean, gives but a faint Adamkiewicz reaction. Therefore the absence of a tryptophan reaction in the autodigestion experiments with the bean cotyledons would not be conclusive evidence of the lack of proteolysis. The use of the Millon's reaction for tyrosin and the biuret reaction for albumoses and peptones would have shown the presence of the products of peptonization or proteolysis had they been present. Still more conclusive evidence that the enzyme in question does not act on the unaltered proteids of the cotyledons is afforded by the more thorough study of the enzyme.

Beans were allowed to germinate for four days and the cotyledons separated and ground in a mortar to a paste. Forty grams of this pulp were extracted with 200^{cc} of water and 5^{cc} of toluol. The extract was removed from the tissue residue by straining through fine muslin, and, since phaseolin is soluble in water and the salts of the seed, this milky looking fluid was rich in proteid matter. The 200^{cc} of extract were divided between two flasks and one of the portions boiled. After digesting for seventeen and one-half hours, a comparative quantitative estimation of the amount of nitrogen in the proteid decomposition products was made in the following manner. Fifteen cubic centimeters of each digestion fluid were mixed in a dry beaker with 15^{cc} of tannic acid reagent (7 per cent. of tannic acid dissolved in 2 per cent. acetic acid) and the precipitates removed by filtration. Nitrogen determinations were made in duplicate by the Kjeldahl method on 5^{cc} of each filtrate. The amount of standard acid required to neutralize the ammonia formed by the sulphuric acid treatment was, in the unboiled digestion, 0.7^{cc}; in the boiled digestion 0.5^{cc}. The digestions were allowed to proceed for two

[17] OSBORNE and HARRIS, *loc. cit.*

days longer and the tests repeated. This time the unboiled digestion required 0.5^{cc} of acid; the boiled digestion required 0.5^{cc}.

This experiment was repeated, using different conditions of reaction to make sure that such conditions played no part in the results previously obtained. Fifty grams of cotyledons from seven-day etiolated bean seedlings were comminuted and an extract prepared as before. This was divided between five flasks as follows:

No. 1—50^{cc} extract.
No. 2—50^{cc} extract—boiled.
No. 3—50^{cc} extract + 0.14^{gm} citric acid ($= \frac{N}{25}$ acid).
No. 4—50^{cc} extract + 0.106^{gm} Na_2CO_3 ($= \frac{N}{25} Na_2CO_3$).
No. 5—50^{cc} extract + 2^{gm} Witte peptone.

Toluol was added in equal amount to all the flasks. The quantitative estimations were made in the same manner as in the last experiment except that 10^{cc} of each filtrate were used for an analysis instead of 5^{cc}. In place of a boiled control for no. 5, a determination was made at the start of the experiment, when it appeared that 1.4^{cc} of standard acid were required to neutralize the ammonia formed from the nitrogen compounds in 10^{cc} of the filtrate from the tannic acid precipitate. After three days' digestion quantitative determinations were made on all.

No. 1—required 1.3^{cc} of standard acid.
No. 2—required 1.0^{cc} of standard acid.
No. 3—required 1.5^{cc} of standard acid.
No. 4— } analyses lost by breaking of the apparatus.
No. 5— }

Three days later the tests were repeated.

No. 1—required 1.4^{cc} standard acid.
No. 2—analyzed 5^{cc} of digestion fluid = to 10^{cc} of tannic acid filtrate; required 4.3^{cc} standard acid.
No. 3—required 1.4^{cc} standard acid.
No. 4—required 1.1^{cc} standard acid.
No. 5—required 6.5^{cc} standard acid.

The digestion fluid of no. 5 at the close of the experiment gave a strong tryptophan reaction. The variations among the digestions, except no. 5, are within 0.5^{cc} of standard acid; in such a method

these differences cannot be held to show the presence of a trypsin-like enzyme, especially when we see the change in the digestion containing Witte peptone which at first required 1.4^{cc} of acid for 10^{cc} of filtrate and at the close 6.5^{cc}.

Being convinced by the experiments tried that the resting and germinating bean contained an ereptase, I attempted to separate it from the mass of other materials in the seed extracts.

One hundred and fifty grams of bean meal were extracted for five hours with 375^{cc} of water with the addition of toluol and chloroform. The fluid was strained and pressed out through cheese cloth, the residue mixed with 150^{cc} of water and this likewise removed. The total extract, amounting to 450^{cc}, was allowed to settle over night to get rid of the starch, etc. by sedimentation. The next morning the supernatant fluid was siphoned off and filtered three times through pulp filters, yielding 365^{cc} of an opalescent fluid. This was half saturated with ammonium sulphate by adding an equal volume of saturated ammonium sulphate solution. The flocculent precipitate *A* was removed by filtration, and to the filtrate saturated ammonium sulphate solution was added to make two-thirds saturation. Precipitate *B* was likewise removed and ammonium sulphate added to three-quarters saturation. With this concentration of the salt nothing but a turbidity resulted; accordingly ammonium sulphate in substance was added to saturation, yielding the precipitate *C*. A small portion of each precipitate was placed in a test tube and mixed with Witte peptone and water; after standing over night in the incubator the fluids were tested for tryptophan. The results showed that *A* contained sufficient ereptase to cause a strong tryptophan reaction, *B* a weak one, and *C* none at all. Each precipitate was dissolved in water so far as possible, and the turbid solution placed in dialyzing bags and dialyzed until practically free from sulphates. In *A* a considerable amount of phaseolin was precipitated on the sides of the dialysis; this was filtered off, washed with water, and both the clear filtrate and the globulin tested with Witte peptone. It was found that the ereptase had remained in solution and had not been carried down with the precipitated proteid. The solutions of the precipitates *B* and *C*, after dialysis, were found to have respectively a weak action on Witte peptone and no action at all.

The ereptase solution obtained from *A* gave a weak biuret reaction and a faint coagulum on heating. Attempts to precipitate the enzyme by means of uranium acetate according to Jacoby's method failed; no precipitate was obtained except in acid solutions in which the enzyme was destroyed. The ereptase was tested with crystallized edestin from hemp seed, crystallized excelsin from the Brazil nut, phaseolin from the bean, boiled fibrin, and Witte peptone. The native proteids were all unattacked and the Witte peptone was actively digested.

An ereptase solution was prepared from 1500^{gm} of bean meal by extraction with water, filtering the extract, half saturating the solution with ammonium sulphate, and dialyzing the solution obtained from this half saturation precipitate. A perfectly clear solution resulted after filtering off the phaseolin precipitated by dialysis, and this was dried at 40 to 50° C., yielding 2.9^{gm} of a golden yellow powder. This powder was found to be active in digesting Witte peptone, although it seemed that the purification and drying had considerably diminished the power of the enzyme. This protease preparation was tested with some of the fractions of Witte peptone which had been separated by PICK'S[18] method and with a preparation of phaseolin. The first experiment was carried out using the secondary protease preparations without the removal of the ammonium sulphate left adherent by the method of separation.

One and one-half grams of the enzyme preparation mentioned above were dissolved as completely as possible in 300^{cc} of water, 35^{cc} of this solution measured into each of five flasks, and 2^{cc} of toluol added. Proteids were added as follows:

No. 1—1^{gm} of phaseolin.
No. 2—1^{gm} of proto-proteose.
No. 3—1^{gm} of hetero-proteose.
No. 4—1^{gm} of secondary albumose *A*.
No. 5—1^{gm} of secondary albumose *B*.

After shaking, 15^{cc} were removed from each flask, 15^{cc} of tannic acid reagent added, and the precipitates filtered off. Analyses of 10^{cc} of each filtrate were made in duplicate:

No. 1—required 0.7^{cc} of standard acid.
No. 2—required 0.6^{cc} of standard acid.

[18] PICK, Zeitschr. Physiol.-Chem. **24**: 246. 1898.

No. 3—required 0.8^{cc} of standard acid.
No. 4—required 10.3^{cc} of standard acid.
No. 5—required 7.6^{cc} of standard acid.[19]

After six days in the incubator the quantitative estimations were repeated.

No. 1—required 0.6^{cc} standard acid = gain of 0.0^{cc}.
No. 2—required 1.9^{cc} standard acid = gain of 1.3^{cc}.
No. 3—required 1.3^{cc} standard acid = gain of 0.5^{cc}.
No. 4—required 10.9^{cc} standard acid = gain of 0.6^{cc}.
No. 5—required 9.2^{cc} standard acid = gain of 1.6^{cc}.

The results in the case of the phaseolin, the proto-proteose, and the deutero-albumose *B* are well defined; those with the hetero-albumose and the deutero-albumose *A* were not sufficiently marked to lead to a definite conclusion.

A second experiment was tried with the four proteose preparations after the ammonium sulphate had been removed from the two deutero-proteoses. In these trials, instead of using the ereptase preparation used in the previous experiment, an extract was made from some bean meal and filtered several times through pulp filters until it was nearly clear. Of each of the four proteoses 0.125^{gm} was dissolved in 25^{cc} of water. Ten cubic centimeters of the bean extract were placed in each of eight test tubes and four of the portions boiled. Ten cubic centimeters of each proteose solution were added to a boiled, and 10^{cc} to an unboiled, portion of the extract. Nine drops of toluol were added to each tube, the tubes corked, shaken, and kept in the incubator for sixty-five hours. At the expiration of that time the contents of all the test tubes were boiled and filtered. Of each filtrate 10^{cc} were removed to a clean test tube, 5^{cc} of a 10 per cent. sodium hydroxide solution added, and then dilute copper sulphate to the maximum biuret reaction. After standing for several minutes the strengths of the biuret reactions from the boiled and unboiled digestions of the different proteoses were compared. The reactions showed that all of the proteoses were attacked by the enzyme. The portions of the filtrates not used in the comparative biuret reactions were employed to test for tryptophan. The unboiled digestions with proto-proteose, hetero-proteose, and deutero-proteose *A* gave

[19] The ammonium sulphate in the last two proteose preparations accounts for the high nitrogen in the filtrates from the tannic acid precipitates.

distinct tryptophan tests, that with deutero-proteose *B* was very faint; none of the boiled digestions showed any trace of tryptophan.

In summing up the results of the experiments described above, we may note that evidence of the presence of a protease has been obtained in the leaves of the spinach and cabbage, the blossoming heads of *Daucus Carota* and the developing seeds of that plant, in the leaves and unripe seeds of the chestnut, the etiolated seedlings of *Phaseolus Mungo*, the seeds and seedlings of *Cucurbita maxima*, and the seeds of *Cucurbita Pepo*. In the case of *Phaseolus vulgaris*, experiments with all stages of the germination of the seed have shown that the cotyledons always contain an enzyme of the erepsin group, and at no time can any evidence of the presence of an enzyme capable of attacking the proteids of the seed be obtained. This ereptase may be removed from the bean extracts by half saturation of the solution with ammonium sulphate. On dissolving this precipitate in water and dialyzing the solution free from sulphates, the enzyme is obtained in a solution which is perfectly clear and gives but feeble reactions for proteids. A preparation of the enzyme may be obtained by drying this solution at a temperature below 50° C. The enzyme acts on the proto-proteose, the hetero-proteose, and the deutero-proteoses, separated from Witte peptone; it is quite inactive on phaseolin of the bean, excelsin of the Brazil nut, edestin of the hemp seed, and boiled fibrin.

There can be no doubt but that the large proteid store in bean seeds is utilized in germination, and in all probability this utilization is preceded by cleavage to the amido-acids, hexon bases, etc. What effects this cleavage, and what part, if any, the ereptase plays, are unsolved problems. It is likewise unknown whether or not this enzyme occurs in other parts of the plant or whether enzymes of the trypsin type are present in certain tissues.

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DEVELOPMENT OF SPORANGIUM IN BOTRYCHIUM.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXI.

IRA D. CARDIFF

(WITH PLATE IX)

THE present work upon Botrychium was taken up with the purpose of investigating what was thought by some to be septation caused by sterilization of mother-cells in the sporangium of *B. ternatum*. Both *B. ternatum* and *B. virginianum* were examined, and the two species were found to be essentially the same in their sporangial development. The material of *B. virginianum* was collected near Woodville, Indiana, March 14, 1904; that of *B. ternatum* was collected near Sullivan, Ohio.

The early stages in the development of the Botrychium sporangium were studied by BOWER (1), CAMPBELL (2), and GOEBEL (5), but apparently none of them studied the interesting later stages. In the present work, though a number of preparations were examined, no special attention was given to the early development further than to confirm the studies of the above mentioned investigators, namely, that the sporogenous mass originates from a single hypodermal archesporial cell. CAMPBELL (2) says that the later divisions in the archesporium do not follow any definite rule, but take place irregularly. This does not accord with my observations, the divisions in *B. ternatum* and *B. virginianum* taking place with great regularity. CAMPBELL implies that his *fig. 129, C*, which shows no regularity in cell-arrangement, is in the mother-cell stage. If this is true, it indicates an unusually small output for a Botrychium. HOLTZMAN (6) made some observations upon the later development of the sporangium of Botrychium, but BOWER (1) thinks that the sequence of segmentations, as shown in HOLTZMAN'S *figs. 3–6*, is not sufficiently intelligible, and should be investigated afresh. He thinks that HOLTZMAN'S description suggests a mode of segmentation more clearly analogous to that in leptosporangiate ferns.

In the sporangium of Lycopodium, the sporogenous mass is divided into blocks of cells, each block apparently being the descendant of a single archesporial cell. The blocking here is evidently related to a multicellular archesporium. In the Botrychium sporangium there occurs a blocking of the sporogenous mass that must have a different cause, as the archesporium is unicellular. The single hypodermal archesporial cell divides usually anticlinally, then periclinally (*fig. 1*). Regular divisions then follow, as shown by *fig. 2*. At the stage shown in *fig. 2* the wall is six to eight cells in thickness, including the tapetum; and the extremely glandular character of the two inner layers of cells indicates that they are definitely set apart as tapetum. In the tapetum at this stage walls are formed both periclinally and anticlinally. The divisions in the sporogenous mass occur with remarkable regularity. The position of the original walls, from stages shown in *figs. 1* and *2*, are still perfectly apparent up to the spore mother-cell stage (*fig. 3*). At this stage the sporogenous mass has somewhat the appearance of the spermatogenous mass in a bryophytic antheridium, except for the size of the cells and the character of the nuclei. By the time the mother-cell stage is reached, the tapetum has become four or five cells thick, is quite glandular in appearance, and there is no further evidence of mitotic division. The sporangium wall-cells adjacent to the tapetum have commenced to collapse as a result of the drain upon them by the tapetum.

Probably the most common and most primitive method of nourishment of mother-cells is by abortion and absorption of a portion of the mother-cells to form a diffuse tapetum, as in Equisetum. Another method is by the formation of sterilized tracts from potentially sporogenous tissue through which material may be conducted to the interior of the sporangium, as the trabeculae of Isoetes or the septations in the microsporangia of *Lemna minor*.

In the sporangium of Botrychium, no sterilization of either kind was found, every mother-cell functioning. So far as I was able to determine, division up to the mother-cell stage is simultaneous throughout the sporogenous mass, yet the original blocks (*fig. 3*) of sporogenous cells still remain perfectly distinct. As the mother-cell enters upon the synapsis stage, the original walls or wall separating

the blocks is apparently thicker, and by the time the spirem is formed the blocks have begun to separate. This separation takes place in the order in which the original walls were laid down in the archesporium and young sporogenous mass (*figs. 3, 4, 6, 7*). At about prophase the mass has separated into at least sixteen (in section) distinct and separated masses (*fig. 6*). "About" prophase is used, since with the separation of the blocks differences in stages of division begin to appear, so that the cells throughout the entire sporogenous mass are not in the same stage, though those in the same block are always in the same stage.

The progressive separation into smaller blocks continues in the same manner in which the first blocks were formed. It takes place along the same lines and in the same order in which the earlier walls were laid down. Whatever stimulus caused the simultaneity of division during the early life of the sporangium seems to have been interfered with here by the cleavage into disjoined blocks, for in the same sporangium the blocks are in different stages of division, it being quite common to find four or five stages. For example, in three adjacent blocks, the cells of one were in early metaphase, of another in telophase, and of the third in anaphase. In another case blocks were found in the same sporangium varying from metaphase of first to metaphase of second division; and it is very common to find them varying from metaphase of first to prophase of second division. One sporangium was found in which all the cells of one-half the entire sporogenous mass were in metaphase, while those of the other half were in telophase. All of the sporogenous tissue throughout its entire development appears in a vigorous and perfectly normal condition.

What may be the cause of this retention of their individuality by the developing sporogenous cells is difficult to say. In regard to bryophytic antheridia this phenomenon has been explained as due to the independent development of the original spermatogenous cells; and there is no further blocking of the spermatogenous mass after the periclinal walls which cut off this mass from the antheridial wall layer have been formed. The difference in rate of development of these blocks has been attributed to differences in food supply, or to some purely physiological cause. It seems that as much might be

said of the blocking in a Botrychium sporangium; though each division, from the very first almost to the formation of the mother-cell, separates masses which retain their individuality throughout their further development. The rate of growth, however, and apparently the food fupply are absolutely the same for all the blocks up to the mother-cell stage. It would seem that in such large sporangia some blocks or cells would be more favorably located than others with reference to food supply or conditions of growth, and there would be greater growth on the part of some regions than others, thus causing irregularities in the arrangement of cells, such as is found in most sporangia, instead of the very regular arrangement in those of Botrychium. Therefore, the above mentioned physiological explanation in regard to bryophytic antheridia is not entirely satisfactory.

What may be the exact cause of this progressive separation of the sporogenous mass along lines where the earlier walls were laid down is impossible at present to say. To me, the most reasonable explanation which can be offered is that the middle lamellae of the walls are acted upon by an enzyme at a time when the sporogenous mass is greatly in need of food. As the lamellae grow older their composition may change, so that they are more easily digested than those more recently formed, thus effecting a progressive separation of the sporogenous tissue. As the blocks become separated, and wholly or partially surrounded by the tapetum, some blocks will of necessity be under slightly different conditions of osmotic pressure or chemical stimulation than others, thus bringing about differences in the rate of their development.

In Botrychium the tapetum is derived from the wall, and absolutely no contribution is made to it from the sporogenous tissue. By the time the sporogenous mass has reached as much as sixty-four cells, the tapetum is clearly delimited from the sporangial wall and is two cells in thickness. From this time on periclinal divisions take place rapidly until the spore mother-cell stage is reached, when the tapetum is four or five cells in thickness and very glandular in appearance. At the first separation of the blocks (*figs.* 3 and 4) in the sporogenous mass—about the synapsis stage—the cell walls of the inner layer of tapetal cells begin to disintegrate. Some of these inner cells are at this time binucleate, while the nuclei of the next one or two layers

of cells are in process of division amitotically, and can be found in all stages. All the tapetal nuclei have now increased much in size, those of the young tapetal cells being 8μ in diameter, and those of the later stage 15 to 20μ. As the blocks of sporogenous cells continue to be formed and more widely separated, the tapetum commences to grow rapidly. The number of nuclei increases greatly, as well as the volume of the cytoplasmic mass in which they float. This pushes inward between the blocks with quite a regular outline (*fig. 6*). At prophase of the first division of the mother-cell, a section of the sporangium shows that these tapetal plates have extended almost across the sporogenous mass between the first formed blocks, and have commenced to grow inward between the blocks of later formation (*fig. 6*). This rapid tapetal growth continues pushing thinner and thinner plates between the smaller blocks as they are formed (*figs. 7, 8, 9*), making a network which finally invests the individual tetrads, or groups of two or four tetrads (*fig. 9*); and at last the spores separate and float in this tapetal mass. The original thicker plates of tapetum may often be found after the spores are completely formed.

As this excessive tapetal growth takes place, the cell walls of the original tapetum break down successively from the inner layers outward, until at metaphase of the mother-cell the walls of only the outermost layer of tapetal cells remain. By the time anaphase of second division (*fig. 8*) is reached, the last walls of the tapetal cells have entirely disappeared, and the inner cells of the sporangial wall begin to take on tapetal characters; especially is this true of the nuclei which resemble tapetal nuclei very closely (*fig. 9*). The cell walls, while they collapse, have not been found to disintegrate entirely, as in the case of the true tapetum. In fact, there is no reason why these inner layers of sporangial wall might not be called tapetum.

Probably the most interesting feature of the development of the Botrychium sporangium is the unusual growth of the tapetum. As before mentioned, it increases greatly in volume and in number of nuclei, yet not a wall is formed anywhere, though it was stained especially for walls. The nuclei are found in all stages of amitotic division (*figs. 5, 8, 9*), but no evidence of mitosis is found. They take stains strongly, are exceedingly large, as mentioned above, and have an unusually thick nuclear membrane. That the nucleus

bears an important relation to the metabolic processes of the cell is too well recognized to need discussion, and this enlargement of nuclei, or increase in nuclear surface, is undoubtedly in response to the increased demand for nourishment on the part of the sporogenous tissue at this stage.

It has been noted frequently that the ovarian follicle cells of arthropods have many large nuclei which divide amitotically, and this has been explained by FLEMMING (4) and CHUN (3) as being a means of securing more rapid metabolic processes between cytoplasm and nucleus through the use of a larger nuclear surface. Our reliable knowledge of the process of amitosis is so meager that one can at this time venture only a tentative explanation of the nuclear behavior in the Botrychium tapetum. Whether amitosis can take place more rapidly than mitosis is unknown, but from the mechanics of the two processes, it would seem that the former would be much the more rapid. WILSON (9) considers that all nuclear division is the response to particular stimuli, and is probably incited by local chemical changes, an idea which is confirmed by PFEFFER (8) and NATHANSOHN (7), who were able to produce mitosis or amitosis at will in *Spirogyra orbicularis*. May we not then look upon amitosis in the tapetum as simply an acquired character due to the unusual demand upon it by the fertile tissue for nourishment at a particular period in its development? Walls being unnecessary, the energy of the organism would not be used in forming them.

As the spores commence to separate in the tetrad, the tapetal cytoplasm has entirely filled the sporangium and many of the nuclei have begun to disorganize, though they seem unusually persistent and many are found after the tetrad is fully formed. Later, when the spores are entirely separated and mature, the tapetum disappears.

Thus we have here worked out the problem of nourishment in a large sporangium by a method entirely different from the two formerly mentioned, namely by the preservation of the individuality of sporogenous cells, thus enabling the mother-cell mass to separate easily into regular blocks, and leaving straight open passageways through which a non-sporogenous tapetum grows rapidly, furnishing the required nourishment for the developing spores. As a general rule there are more spores provided for in a sporangium than nutri-

tive conditions will allow, and there are usually two methods by which this difficulty is overcome: by abortion of mother-cells, and by arrangement for a succession of sporangia according to nutritive supply. In Botrychium, however, there is no abortion of mother-cells and very little difference in the stages of development of the different sporangia in a spike. The nutritive supply is equal to the demand of all mother-cells, probably owing, in part, to the slow growth of the plant, and also to the large amount of food material stored in the stem.

SUMMARY.

1. The sporogenous tissue develops from a single hypodermal archesporial cell.

2. As the successive sporogenous cells are formed, each retains its individuality throughout the development of the sporogenous tissue.

3. Divisions in the sporogenous tissue are simultaneous up to the mother-cell stage.

4. Beginning with the mother-cell stage, the sporogenous mass separates successively into blocks of cells in the same order in which the earlier cells were formed.

5. The blocks develop independently, and at a different rate in the same sporangium, though all cells of one block develop at the same rate. Whatever stimulus caused the simultaneity of division in early sporogenous tissue, is interfered with by separation of the cells into disjoined groups.

6. The progressive separation of the sporogenous mass is probably caused by the digestion of the middle lamellae.

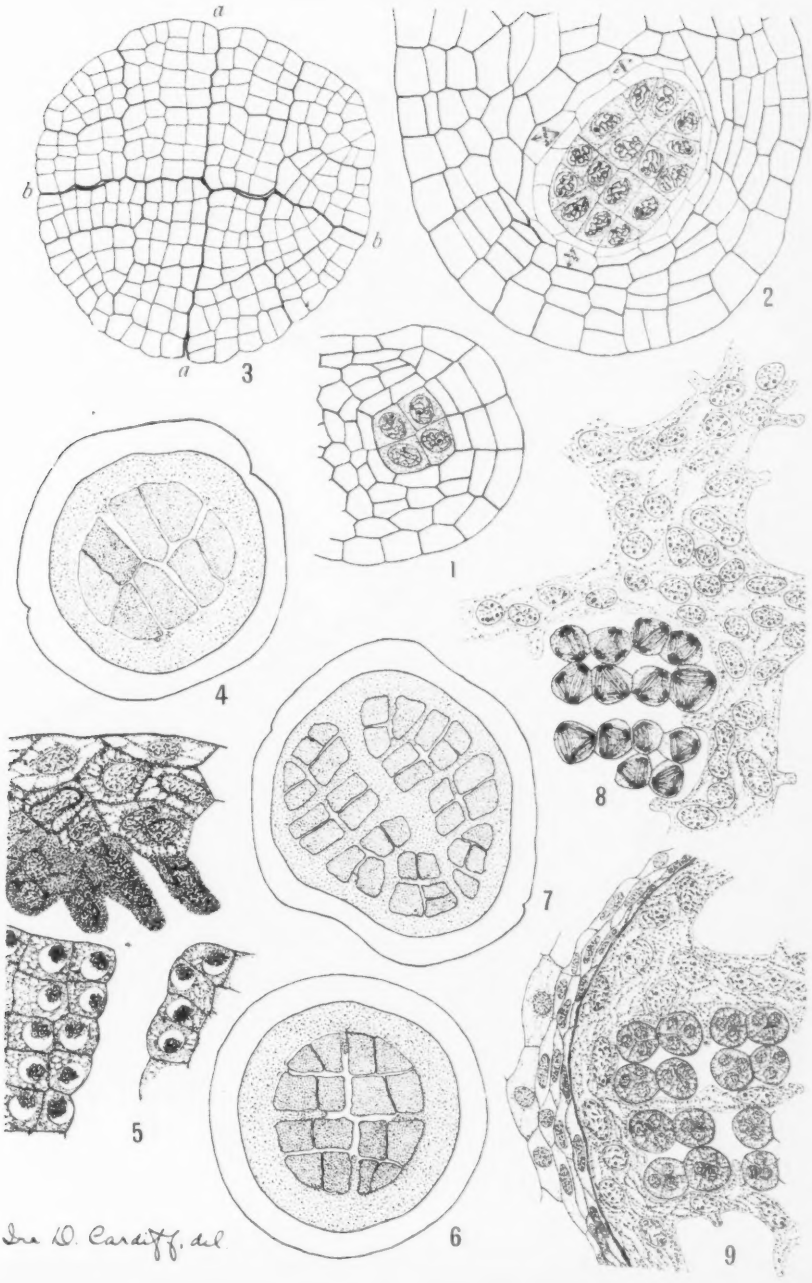
7. All mother-cells produce spores.

8. The tapetum is of non-sporogenous origin.

9. With the separation of mother-cell groups, the tapetum grows rapidly between them without the formation of walls; the nuclei increasing greatly in size, and dividing amitotically.

10. The problem of nourishment in large sporangia may thus be solved by individual development of sporogenous cells, by their later separation into regular groups, and the rapid growth of tapetum between them.

11. The nuclei of the old tapetum are four times the size of those in younger stages of its development.



CARDIFF on BOTRYCHIUM

12. Amitotic division and increase in size of nuclei are both devices for rapidly increasing nuclear surface, thus effecting a larger increase of metabolic products.

I am indebted to Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN for criticism and advice.

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EXPLANATION OF PLATE IX

All figures were made with Zeiss objectives and oculars and Bausch Lomb camera lucida. The original drawings were reduced one-half in reproduction.

FIG. 1. Young sporangium. × 900.

FIG. 2. Sporogenous tissue of thirty-two cells; tapetum two cells thick and divisions taking place both periclinally and anticlinally. × 900.

FIG. 3. Sporogenous tissue in mother-cell stage showing regularity in cell arrangement; the two original walls, *a* and *b*, beginning to separate. × 400.

FIG. 4. Sporangium showing sporogenous tissue separating into blocks along the original walls. × 150.

FIG. 5. Portion of *fig. 4;* mother-cells in synapsis; commencement of tapetal growth. × 900.

FIG. 6. Sporangium showing sporogenous tissue separating along original walls shown in *fig. 2;* plates of tapetum extending between earlier formed blocks. × 150.

FIG. 7. Sporangium showing sporogenous tissue separated into blocks in each of which the cell division is simultaneous. × 150.

FIG. 8. Portion of sporangium showing separation of the forming tetrads, also the large increase in the number of tapetal nuclei. × 900.

FIG. 9. Later stage than shown in *fig. 8*. × 900.

PHYSIOLOGICAL PROPERTIES OF BOG WATER.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXII.

BURTON EDWARD LIVINGSTON.

(WITH THREE FIGURES)

ALTHOUGH it has been shown (1) that water from the bogs of the northern United States contains solutes to such a very small amount that its osmotic pressure is generally not appreciably above that of the river swamps and lakes of the same region, still the generally observed xerophilous character of bog vegetation may be due to small amounts of dissolved substances of such nature that they affect the plants chemically through toxic stimulation. Having found that metallic ions affect the vegetative growth of the polymorphic Stigeoclonium with which the author has been experimenting for some time (2), and that the effect thus produced is identical with the response of the alga to high osmotic pressures, it was suggested that this alga might be used as an indicator in a study of the physiological properties of bog waters. In accordance with this suggestion, natural waters of a number of different types were collected in bottles, filtered through filter paper, and tested as culture media for the alga. The result of these tests is, briefly, that many bog waters act upon the plant like poisoned solutions. Details of the work are given in the following pages.

The form of Stigeoclonium here used has already been shown (2–6) to take either of two forms according to the medium in which it is grown. In solutions of low osmotic pressure at ordinary temperatures it assumes the form of branching filaments composed of cylindrical cells. In the same solutions at a temperature slightly above the freezing point of water, and at ordinary temperatures in solutions similar to these but poisoned with certain metallic salts, as well as at ordinary temperatures in solutions of high osmotic pressure, the plant takes the palmella form, in which the cells are spherical or nearly so and lie in the medium singly or in irregular groups. If

filaments are taken from the conditions which favor their growth and are placed in those favoring the palmella form, their cells round off and partially or completely separate, they begin to divide slowly in all planes, and the result is the typical form for these conditions. A return to the conditions for filaments is followed by the resumption of that form, partly by the growth of filaments directly from the palmella masses, and partly by the production of zoospores which germinate to form new filaments. Zoospores are not produced in solutions of high pressure, and they fail to germinate in solutions which produce the palmella form, though they are produced in the cold and in poisoned solutions.

The waters tested in these experiments are in large part the same as were used in the determination of the osmotic pressure (1). The work was carried on partly at the New York Botanical Garden and partly at the University of Chicago, and extended from June 1902 to January 1905. The cultures were made in the manner described for water cultures of this plant in the author's earlier papers.

Since a number of authors have loosely attributed SCHIMPER'S "physiological dryness" (7) of bogs to acidity, titrations of most of the natural waters here employed were made with $n/100$ KOH solution, using phenolphthalein as indicator.[1]

Data from the experiments are given in the following table. In the column of responses, F—F denotes that the filamentous form persisted as such when placed in this particular water. F—P denotes that the original filaments became palmella and that no new filaments were produced. F—$\frac{1}{2}$P denotes that filaments persisted, but that there was also a marked production of palmella. A number of cultures were made with each of the waters, and the result is the general one for all. The cultures were continued for two to four weeks. The acidity data are given in terms of normal acid and the pressure in terms of millimeters of mercury at 25° C.

From the table it is seen at once that in some of the waters the palmella form was produced; in others it was produced in some measure, but filaments persisted; and in still others the filamentous

[1] Of the indicators at hand this was the best, although it is assuredly not perfect for such acids as are probably present in these waters.

DATA FROM THE EXPERIMENTS

Source of water and nature of vegetation	Response of Stigeoclonium	Osmotic pressure of water, mm. of Hg	Acidity of water in terms of normal acid
Drained swamps of Hackensack River, N. J. (river swamps)—			
Average of six samples	F—½P	50	0.00192
Maximum of six samples	.....	..	0.0042
Minimum of six samples	.....	..	0.001
New York City supply, Croton and Bronx Rivers	F—F	50	0.0005
Chicago City supply, Lake Michigan	F—F (see *fig. 1*)	100	Always alkaline, about 0.0015
Grand River, Grand Rapids, Mich.	F—F	100	As last, or more alkaline
Aetna, Ind. (Rhus vernix, Drosera)—			
Average of three samples pressed from black peat	F—½P	100	.....
Miller, Ind. (Larix, Rhus vernix)—			
Average of two samples pressed from black peat	F—½P	50	0.0003
Oconomowoc, Wis.* (typical Larix swamp), ditch	F—P	200	0.0004
Stewart Ridge, Ill., average of three samples pressed from black peat	F—F	150	0.0002
Ann Arbor, Mich.†			
First Sister Lake bog (Larix, Chamaedaphne, Sphagnum)—			
Sample A	F—P (see *fig. 2*)	50	0.0033
Sample B	F—P	440	0.0038
West Lake bog (Sphagnum, Chamaedaphne, Potentilla palustris, Salix)—			
Sample A	F—P	100	0.0026
Sample B	F—P (see *fig. 3*)	150	0.0024
Tom's River, N. J. (Chamaecyparis, Sphagnum, Chamaedaphne, Sarracenia, Oxycoccus)—			
Sample A, pressed from Sphagnum	F—F	170	0.0004
Sample B, beneath Sphagnum	F—P	140	0.00048
Sample C, pressed from black peat	F—P	90	0.0003
Sample D, margin of pond	F—P	40	0.0003
Sample E, ditch	F—P	50	0.0003
Richmond, Staten Island, N. Y. (Alnus, Eriophorum, Sphagnum)			
Sample A, edge of pond	F—P	90	0.0015
Sample B, decayed leaves	F—½P	110	0.0022
Sample C, ditch	F—F	90	0.0008
Sample D, pressed from Sphagnum	F—F	100	0.001

* This water was obtained for me by Dr. H. C. COWLES of this laboratory.

† The samples from Ann Arbor were obtained for me by Dr. H. N. TRANSEAU, of Alma College, Mich.

form persisted without the production of palmella at all. The characteristic forms of this plant in three different waters are shown in *figs. 1*, *2*, and *3*, which are to be compared with previously published figures, and are self-explanatory.

As has been shown in the case of many inorganic poisons (5), the production of the palmella form is sometimes accompanied in these waters by a stimulation of zoospore production. Usually, however, the swamp water acts more like low temperatures, producing the vegetative response without either accelerating or retarding the reproductive activity.

The palmella response in certain of these waters may be due,

a priori, to either of two sets of factors, the osmotic pressure of the solution or the chemical nature of the solutes. Since the experiments were carried on at room temperature, it is unnecessary to consider low temperature as a possible stimulus.

In the work on the influence of osmotic pressure upon this plant it was found (2, 3) that there is no tendency to form palmella till a pressure of about 1618.6^{mm} of mercury has been attained. Filaments still persist at a pressure of 3237.1^{mm}, but have practically all disappeared at a pressure of 6474.2^{mm}. But no swamp water studied has a pressure at all approaching the lower limit for even the incipient

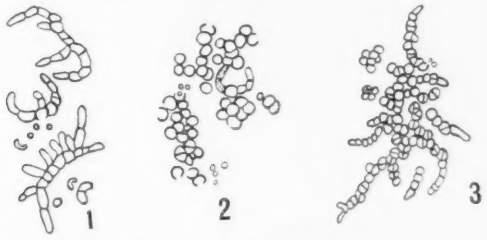

FIG. 1. Stigeoclonium filaments in Lake Michigan water.

FIG. 2. Stigeoclonium, palmella form from filaments, in water from West Lake, Ann Arbor, Michigan, sample A.

FIG. 3. As *fig. 2*, but in water from First Sister Lake, Ann Arbor, sample B.

formation of palmella. Therefore, we are forced to the conclusion that the palmella response in these bog waters is due to the chemical nature of the solutes. The plant grows well as filaments for a long time in distilled water, so that it is impossible to relate the reasons to absence of inorganic salts.

Definite knowledge of the chemistry of bog water is beyond our reach at present. It appears that all bogs are acid to some degree, and there has been a tendency for many authors, *e. g.*, SCHIMPER (7, pp. 4, 8, 657, etc.), to attribute the peculiarities of bog plants to this property. In the author's study of chemical stimulation (5) it was found that nitric and sulfuric acids produce palmella at concentrations of from 0.0001*n* to 0.00006*n*. The natural swamp waters are uniformly more acid than this; therefore, were the acid property of the latter due to either of these mineral acids, we should expect *all* of these waters to produce the palmella response. This is obviously

not the case, and so it seems highly probable at least that the stimulating factor of bog waters is not the hydrogen ion.

Further, a comparison of the acidity figures with the response of the plant in the different waters shows clearly that the former data could not be used as a criterion for the prediction of the latter. This is clearly brought out in the following list, in which the acidity figures are arranged in order of their magnitude, with the responses occurring in the corresponding water placed after each. Alkalinity 0.0015*n*, F; acidity 0.0002*n*, F; 0.0003*n*, F, ½P, P; 0.0004*n*, F, P; 0.00048*n*, P; 0.0005*n*, F; 0.0008*n*, F; 0.001*n*, F; 0.0015*n*, P; 0.0019*n*, ½P; 0.0022*n*, ½P, P; 0.0026*n*, P; 0.0033*n*, P; 0.0038*n*, P. The lower acidities appear to produce both filaments and palmella, the higher ones only palmella. This would seem to indicate that, while high acidity is always accompanied by the presence of the stimulating substances, these substances are not necessarily accompanied by high acidity.

Boiling the stimulating waters for five or ten minutes and then rediluting to the original volume with redistilled water decreases their acidity from 30 to 50 per cent., but appears not to alter their stimulating power. Apparently the active substances are not volatile at 100° C. Diluting the Ann Arbor samples, and also those from Tom's River numbered *2*, *3*, and *4*, with distilled water or with weak nutrient solution, decreases the toxic effect, and this effect practically disappears when the water has been diluted to twice its volume. This is evidence that the stimulating substances are present in extremely small amount.

The relation of the source of these waters and the type of vegetation growing therein to the physiological properties exhibited toward Stigeoclonium throws some light on the general problem of the xerophilous character of bog plants. The drained swamps of the Hackensack valley are not in any sense bogs. In many places, however, are found spots where Sphagnum has taken a foothold in small pools. Eriophorum, Typha, and some other semi-xerophilous plants are also found here. The water samples studied were taken from such places, and the experiments show that they possess the toxic property to a considerable degree. The data for this broad area of swamps are averaged from a large number of samples taken near Englewood and Closter, N. J., as well as from the western slope

above the palisades of the Hudson, where the water is held in the irregular rock basins.

The New York City water, although quite acid, is not active upon the alga. This water is from streams with well-drained river swamp margins. Lake Michigan water and the water of Grand River appear to be identical. Both are somewhat alkaline; neither has any physiological effect upon Stigeoclonium.

The Aetna and Miller swamps are practically alike; both are composed of black peat with no Sphagnum. The general nature of these swamps suggests an intermediate condition between bog and river swamp, leaning toward the former, and the water shows a marked tendency to produce palmella.

The bog near Oconomowoc has Larix, Sphagnum, Vaccinium corymbosum, Oxycoccus, Chamaedaphne, Sarracenia, etc. Its water has a very marked action upon the alga.

The Stewart Ridge swamp is a peat deposit but not a true bog. A few patches of Sphagnum seem to show a tendency in this direction, but the samples tested showed no effect upon the indicator plant.

The swamps of Ann Arbor are as typical and characteristic bogs as the author has seen. The character of their vegetation, consisting of Larix, Drosera, Sarracenia, Andromeda, Chamaedaphne, Arethusa, Calopogon, etc., agrees well with the fact that the water is markedly toxic toward the alga.

The vegetation upon all the vast stretches of lowland about Tom's River is practically alike; these are dense Chamaecyparis swamps, abounding in Oxycoccus, Sphagnum, Sarracenia, Chamaedaphne, etc. All the samples from here, with the exception of one pressed from living moss, produced the palmella response. It appears that the active bodies are more plentiful in the mass of dead material beneath the Sphagnum than in the moss itself.

The Richmond swamp is not a true bog, and yet it contains considerable amounts of loosely-growing Sphagnum, together with Eriophorum, Typha, etc. Here there appears a disagreement between the physiological properties of water samples from different parts. That from the ditch should be the most dilute, and shows no action upon the indicator. That from moss is again harmless; while that pressed from decayed leaves near by shows a marked toxic effect. The pond sample was taken up within a centimeter or two of sub-

merged powdery peat, and should be saturated or nearly so with any slightly soluble substances contained therein. Its active property is very marked.

From the last eight paragraphs it seems clear that the stimulating substances with which we have been dealing are present in swamp waters to an extent roughly proportional to the xerophilous character exhibited by the swamp vegetation. It is possible that the factor in such bogs which prevents the growth of plants other than xerophilous ones may be these unknown toxic bodies. They act upon Stigeoclonium in much the same manner as do drying media. Perhaps ordinary plants are affected by these substances with the same end result as though they were in a truly dry soil. If this be true it becomes easy to see how plants whose protoplasm is naturally adapted to dry situations may alone be able to thrive in these bogs.

The behavior of this alga toward dryness, cold, and bog water are quite parallel with results obtained by TRANSEAU (8) with *Rumex acetosella*. This author found that in dry mineral soil, Rumex produces thickened leaves reduced in size and with revolute margins, while the palisade tissue is very much increased in amount, and the epidermal cells are reduced in size and have thick, cuticularized outer walls. These changes give the plant, which in moist conditions is anything but a xerophyte, a very characteristic xerophilous structure. The same responses are exhibited, to a somewhat less degree, when the roots are kept at a low temperature, and also when the substratum is a bog soil. But when both the last named conditions are allowed to act together the response is the same in direction and amount as in dry mineral soils. The changes occurring in these leaves are very similar in their nature to those just described for Stigeoclonium. From an ecological standpoint, the palmella form of the alga is extremely xerophilous in character, while the filamentous form lies at the other extreme. It appears that we have here two very widely different plants, both of which respond to these various conditions in the same way.

SUMMARY.

The results of this series of experiments are as follows:

1. There are chemical substances, in at least some bog waters, which affect Stigeoclonium as do poisoned solutions and solutions of high osmotic pressure.

2. The responses of this alga to bog water and to cold are as nearly identical with those obtained by TRANSEAU with Rumex, for the same conditions, as the nature of the two plants would permit.

3. The active substances are not directly related to the acidity of the water.

4. Boiling the water decreases its acidity but does not appreciably affect its action as a stimulating agent.

5. The stimulating substances are most markedly present in water from those swamps whose vegetation is most definitely of the bog type. They are absent from river swamps and large lakes; in water from swamps whose vegetation is of a character intermediate between those of the river swamp and the bog, they are present to some degree, their amount being roughly proportional to the extent of the xerophilous character of the vegetation.

6. The stimulating substances here demonstrated may play an important rôle in the inhibition from bogs of plants other than those of xerophilous habit.

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AN APPARATUS FOR OBSERVING THE TRANSPIRATION STREAM.

OTTO V. DARBISHIRE.

(WITH TWO FIGURES)

THERE are, roughly speaking, two sets of experiments by means of which we can investigate the presence of the transpiration stream in small plants. One of these is concerned with the root of the plant and with the demonstration of the phenomenon known as root pressure. The other set deals with the shoot and the suction exerted by it on the water which is being absorbed by the root.

We all know what happens when we fix some kind of root pressure apparatus to the stump of a small plant, like a fuchsia for example. Water will soon be pressed out of the stump with sufficient force to raise a column of mercury to a considerable height. We can at the same time attach to the shoot of the very plant used for the root pressure experiment some form of potometer. We will observe suction and a column of mercury can be raised to a considerable height thereby. Do these two separate experiments really—even approximately—show what was going on in the plant at the time the experiment was set up? I think the answer must be no. When the plant in question was cut across, the stump exhibited the phenomenon of pressure, but the shoot that of suction at the same level. It is obviously impossible that pressure and suction should be exhibited by an intact plant at the same level. It must be mentioned here, however, that if the two experiments are set up very quickly, we may find the stump exhibiting suction for a short time. This changes to pressure when the stump is saturated with water. The root may of course become saturated in a few minutes by simply being exposed to water during the setting up of the experiment. We do not therefore get a clear idea of what is going on in the plant, when we isolate the shoot from the root, by attaching to each one a separate apparatus.

In order to learn more about the relation between shoot suction and root pressure it is obviously necessary not to separate the two

portions of the plant entirely, but rather to keep them connected by some continuous bit of apparatus, so that, although the plant be cut in two, the shoot suction may still act on the root or the root pressure on the shoot. I use for this purpose a simple bit of glass apparatus, which I have called a ***pinometer***. It will be necessary first to describe the apparatus; secondly, the way it is set up; and finally, to refer to a few of the experiments and the results obtained.

The pinometer consists of a straight bit of glass tubing (*fig. 1, b–d*), to which is obliquely attached by annealing on one side another short bit of glass tubing (*c–j*). On the opposite side a U-tube with an oblique connection is annealed on (*a–e.*) We have therefore four openings to this part of the apparatus (*a, b, c, d*). The bore of the glass tubing used for the pinometer depends entirely on the thickness of the stem of the plant used. The diameter of the glass tubes should about equal the diameter of the latter. A very convenient plant to use for demonstration purposes is a healthy fuchsia plant, not exceeding two feet in height.

The experiment should not be set up till all the various parts and tools are quite ready. The glass tubing should be cleaned to remove any greasiness, which is often the cause of introducing minute air bubbles into the system of tubes. The air should also be carefully removed from the rubber tubing. The more quickly everything is set up, the more nearly will the results obtained show what was going on in the plant at the time the experiment was made.

When everything is ready, the plant in its flowerpot is put into a bucket of water, so that it is immersed to a few inches above the point at which it is to be cut across. The leaves should not be moistened more than is absoluely necessary. The stem of the plant is now cut across under water in such a way that there is about one inch of stem, above and below the cut, devoid of buds or branches. If the stem has already a complete wood cylinder, the cortex may be removed with a sharp knife for about half an inch above the cut on the shoot, and below on the root.

The lower end of the shoot, without removal from the water, is fixed by rubber tubing to the opening *a*, *fig. 1*, and the portion *a–e* of the pinometer remains full of water, even when removed from the

water, and can be temporarily held by a clamp (*i*) on a retort stand.[1] Next some rubber tubing is slipped over the upper end of the root stump. This bit of tubing also fills with water and the whole flowerpot can now be removed. The end *b* of the pinometer is now quickly fixed to the rubber tubing over the stump. A gauge is securely attached to *c*, and water is poured into *d* from a small reservoir (*fig. 3, r*) till the whole system of tubes is full of water. Mercury is then poured into the outer limb of the gauge, and this causes water to pour out at *d*, to which some pressure tubing has been firmly fixed. When there is enough mercury in the gauge to give the columns sufficient play to rise and fall, the opening at *d* should be closed by a pinch-cock and the experiment is set up. Retort stands and clamps are used for keeping the various parts in position, and a millimeter scale (*k*) can be attached to one or both limbs of the gauge.

Should air make its appearance, it will collect under *d*, if it comes from any part of the plant except the lower end of the shoot. It can be removed from *d* by opening the pinch-cock and allowing water to run in from the reservoir. Should it accumulate under *a*, the shoot must be removed from the rubber tubing attaching it to the glass, and water allowed to enter the pinometer at *d* rather slowly. It will then be running out slowly at *a*, and the shoot is again fixed, the current of water preventing any air getting in.

In any case the removal of air means the opening of the pinch-cock at *d*, and this causes the mercury to go back to its starting point. This can be obviated by inserting a stop-cock between the oblique bit *f–c* (*fig. 1*) and the gauge. I do not however consider this necessary for the apparatus, which as described here is intended chiefly for qualitative and not quantitative observations.

The results obtained with the pinometer depend very much on the point at which we fix it in the plant. I will refer therefore to a few actual experiments, which I hope will show the value, however small, of the simple bit of apparatus just described.

In the first experiment to be described, a pinometer was fixed to

[1] For fixing the plant to the glass tube it is best, if possible, to employ some kind of pressure tubing. The latter can be made secure by tying with string or by employing some kind of clamp. The use of wire is to be deprecated.

the main shoot by cutting the stem a short distance above the lowest lateral shoot (*fig. 1*). After a very short time the mercury in the limb (*g*) of the gauge nearest the plant was seen to rise, as the latter was withdrawing water from the pinometer. As the mercury rises, the pull on the lower end of the shoot and on the upper end of the

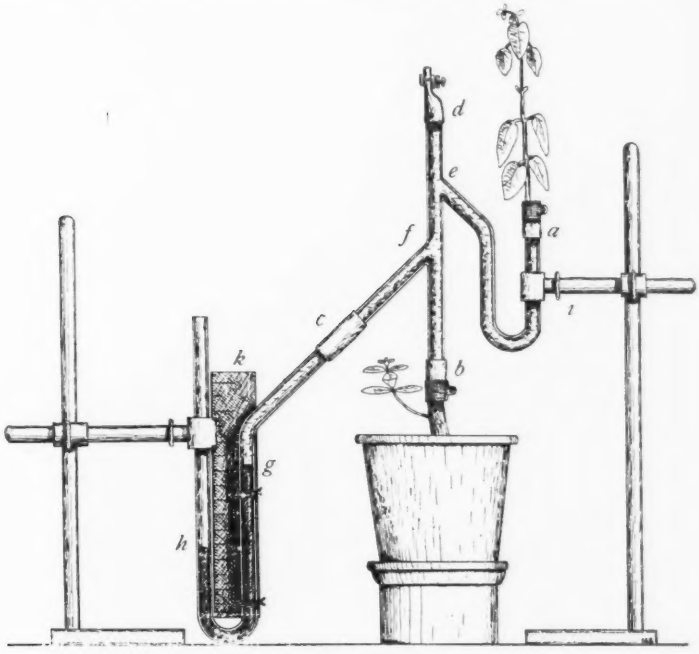


FIG. 1.—A fuchsia plant with one pinometer inserted just above the lowest lateral shoot: *a*, the attachment of the upper shoot; *b*, that of the root stump; *c*, the connection leading to the mercury gauge; *d*, the top opening of the pinometer, closed by rubber tubing and pinch-cock; *g*, the level of mercury in inner, *h*, that in outer limb of gauge; *k*, millimeter scale; the latter indicates shoot suction and the shoot at *a* is wilting; the stump at *b*, under pressure from the root, is flourishing.

root-end of the plant gets stronger, and gradually the leaves of the upper shoot wither. It will be noticed that the leaves of the lowest lateral shoot are quite fresh. Before the upper shoot dies altogether its axillary buds will generally develop and form small leaves, but even these succumb in the end.

In this experiment, therefore, and at the height in question, our plant exhibited shoot suction when tested by the pinometer. A modified barograph recording cylinder, made by NEGRETTI & ZAMBRA, was arranged to record the rise of the mercury in this particular experiment. A burette-float was placed on the mercury in the open end of the gauge (*h*). It was suspended by a fine thread, which ran over a pulley and was attached on the other side to the free end of a lever, the other end of which carried a pen which wrote on the revolving cylinder. The records taken during the first week showed that the water taken in from midday to midnight and that taken in from midnight to midday was in the proportion of three to two. Never did any pressure from the root, during night or day, cause the amount of water taken in to fall below nought.

In a second experiment a pinometer was fixed into a fuchsia plant about one inch above the soil and quite below the lowest lateral shoot. Root pressure manifested itself very soon and the mercury was forced out of the inner limb of the gauge, rising of course as rapidly in the outer one. In this case there was obviously pressure on the cut surfaces of shoot and root. The leaves of the shoot kept fresh as long as the pressure lasted, which was for sixteen days. On the sixteenth day there was a difference in the level of the mercury in the two limbs of the gauge of 20^{mm}. Allowance however must be made for the column of water resting on the mercury in the inner limb of the gauge. After the sixteenth day the pressure was gradually reduced, very probably owing to the root becoming exhausted, its supply of organic food from the green leaves being cut off. The leaves began to wither as the pressure of the root decreased.

In a third experiment two pinometers were employed (*fig. 2*). One was fixed to the fuchsia plant just above the soil and the other just above the lowest lateral shoot. The plant was therefore cut into three parts, the lowest one of which, the stump, was devoid of any lateral shoots. The gauge attached to the lower pinometer (P_1) very soon indicated pressure from the root, that of the upper pinometer (P_2) suction from the two portions of the shoot. We have therefore in this experiment an arrangement by which root pressure and shoot suction can be observed at the same time. The difference in the appearance of the leaves on the two portions of the shoot is very

striking. The leaves of the upper shoot are dead, there being a strong pull on the lower end. The leaves of the middle shoot are flourishing, there being a push on its lower end, although the lower pinometer is separated from the upper one by about two inches of stem only

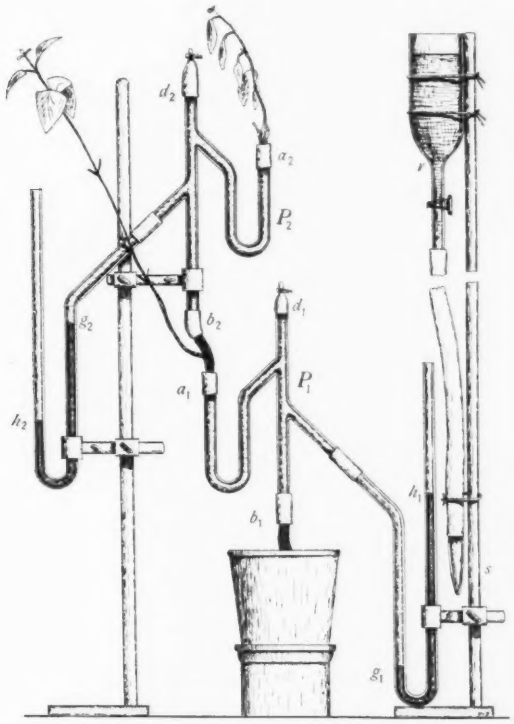


FIG. 2.—A fuchsia plant with two pinometers: the lettering as in *fig. 1;* the lower pinometer (P_1) shows root pressure and its shoot (a_1) is flourishing; the upper one (P_2) exhibits shoot suction, and its shoot (a_2) is wilting; *r*, reservoir of water, *s*, its spout.

(between a_1 and b_2). A fortnight after being set up, a reading for this experiment showed a difference in level of the two columns of mercury of 18^{mm} in the lower pinometer, indicating pressure from the root, and a difference of 20^{mm} in the upper pinometer, indicating suction from the shoot.

An experiment was also made with three pinometers inserted into the stem of a fuchsia plant. The following readings were taken after the experiment had been going on for some time. The lower pinometer showed root pressure with a difference in the level of the mercury columns of 31^{mm}, the middle pinometer showed suction with a difference in level of 85^{mm}, and the upper pinometer suction with a difference of 63.5^{mm}. The figures for the next day in millimeters were 39 (an increase of 8), 127.2 (42.2), and 128 (64.5) respectively. The two lower pinometers were below the lowest branches. In this case, therefore, root pressure could not be observed even up to the lowest lateral shoot. As in all the previous experiments, suction, where observed at all, was maintained day and night, till, owing to the pull on the cut surface of the plant, air made its appearance and the mercury returned to its original level.

The pinometer, as described here, is meant to be of use chiefly for class and demonstration purposes. I wish now to point out what the results obtained by employing it are, that is to say what observations can be made on the transpiration stream.

We can readily see that in our plants, at least, it is never a function of the root to press water up into the leaves. By day and by night the phenomenon of root pressure can be observed only below the lowest shoot. But root pressure is an extremely useful if not a necessary process by which the rise of the water is started. Referring to those of our experiments in which the lower cut surface of the shoot was exposed to pressure, we see that the plants do not suffer much by the stem being cut across. It appears to be necessary that water should be forced into the lowest end of the vascular system; or at least there should be no pull on it. Root pressure, therefore, is not only a symptom of the avidity with which the roots are absorbing water, but it is of importance as assisting in the starting of the transpiration current.

It must be mentioned here, however, that the insertion of a pinometer into a fuchsia plant is in any case a serious thing for the latter. The phenomena of exudation and bleeding seem to cease entirely when the shoot has been severed from its connection with the root. This statement holds good whether the gauge of the pinometer shows pressure or suction. The fact that at different heights in the plant

we get readings which at least show that the pressure may vary very much in even two or three inches of stem, is a proof, I think, that the "atmospheric pressure" theory of the rise of water is not correct.

When we get away from the root we find the phenomenon of shoot suction manifesting itself. In fact, as already pointed out, shoot suction generally seems to be stronger than the pressure from the root at any point except just above the root. That is to say, the shoot is able at any time to take in more water than can be supplied by the root. Numerous experiments show that this is the case in winter and summer, day and night, in the plants I experimented upon.

Which part of the plant is exerting this suction? I have spoken here of shoot suction, but it is possible to split up this part of the transpiration stream into two distinct processes, namely the leaf suction and the wood lift. There is not much mystery about the former. We can understand how the water is removed from the finest endings of the vascular system. If, however, we remove the leaves from a shoot which has been attached to a pinometer or even cut off the upper part bearing leaves, leaving only a short leafless shoot stump, we still get water rising in the wood and exerting a pull on the gauge of the pinometer. The activity of the leaves simply removes the water from the top of the vascular system, and this water is replaced by a process going on in the wood.

I am mentioning this simply to show that in the experiments with the pinometer the taking in of water by the shoot is not a phenomenon of leaf suction but one of wood lift. The force of the wood lift is very great; it is generally greater than the root push. But, as our experiments show, it cannot act efficiently for any length of time if the lowest end of the vascular system is exposed to a pull from below. It is as yet not known how the wood lift acts. It is therefore all the more necessary to make as many observations as possible on this process. I think the pinometer does make it possible to observe at least one property of this water current. It is continually in a state of what is known as "negative pressure." A natural result of this is that the air, which the water absorbed by the plant contains under ordinary atmospheric pressure conditions, escapes from the water when inside the plant. Over and over again my experiments have been brought to an end by air collecting, generally under the shoot. Air-bubbles

are bad for the conducting of the experiments, and the more they are kept out the more water is absorbed by the plant when attached to a pinometer. It is obvious that the plant must also guard against the accumulation of air in the column of water which fills the vascular system. No doubt this will in part account for the peculiar structure of the wood elements. Do the latter bear any relation in their structure to the nature of the water generally found in the localities in which the plant grows?

The form of pinometer mentioned in this paper is intended, as already mentioned, essentially for demonstration purposes. It is possible by its use to observe, more clearly than hitherto, the relation between shoot suction and root pressure. Owing to the preliminary nature of this paper I have refrained from giving any lengthy readings taken during the experiments. Their value in any case would not be very great, being taken with the simple form of pinometer here described. A more elaborate bit of apparatus is therefore in course of construction, by which any air making its appearance in any part of the pinometer is removed automatically at regular intervals without altering the conditions of pressure inside the system of tubes. Furthermore, it is connected with an automatic recording instrument, in which the difference in the height of the two mercury columns will be reduced as much as possible.

The nature of this paper, I hope, will excuse my having made no reference to any literature. It was not my intention to discuss the old or bring forward a new theory with regard to the rise of water. We still have the suction by the leaves, the pressure by the root, and the as yet little understood lifting of the water by the wood. In writing this paper, it was my object to give an account of a very simple bit of apparatus, by means of which various phenomena connected with the transpiration stream in small plants could be readily observed. I hope later on to be able to publish some more detailed observations on the properties of the wood lift, taken by more elaborate instruments.

Diagrams illustrating the working of the pinometer were shown at the last Cambridge meeting of the British Association.

UNIVERSITY OF MANCHESTER, ENGLAND.

BRIEFER ARTICLES.

POLYEMBRYONY IN SPHAGNUM.

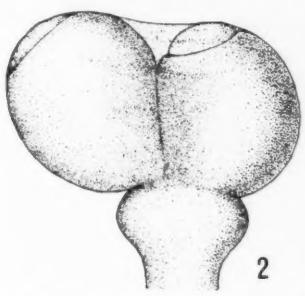
(WITH THREE FIGURES)

DURING July 1904, while collecting sporophytes of Sphagnum for class use, the writer discovered the peculiar specimens described in this note. The particular species of Sphagnum has not been determined, but it is a large variety which grows abundantly in the swamps around Tower, Minnesota.

1

Among the thousands of sporophytes handled, five cases were found where in two archegonia at the end of the same branch the oospheres had been fertilized and each oosperm had developed into a normal sporophyte. In these specimens each capsule was enclosed by its separate calyptra and the haustra of each two sister sporophytes were inserted side by side in the bulbous end of the pseudopodium (*fig.* 1).

Two cases were also found where two sporophytes had developed in the venter of one archegonium (*fig.* 2). They were more or less distorted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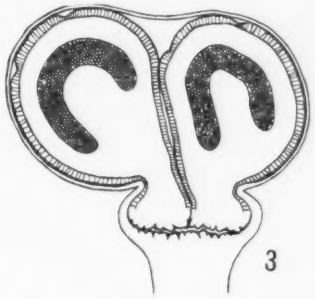
3

and modified by mutual pressure, but each presented the essential structure of a normal sporophyte (*fig.* 3). When studied in sections, the tissue of

each sporophyte appeared to be entirely independent of that of its twin except in the loose tissue forming the base of the haustra, where the line of separation was not clearly recognizable in all sections. In the region of contact, the epidermis, while quite distinct, was less strongly developed than on the free faces. Since the sporophytes were nearly mature at the time of collection, it was impossible to determine the conditions which led to the production of twins. It is probable that two functional oospheres were produced in the venter of each archegonium, although it is not impossible that twinning took place through an early branching of the young protocorm.—HAROLD L. LYON, *University of Minnesota*.

ADENODERRIS, A VALID GENUS OF FERNS.

(WITH TWO FIGURES)

THE genus Polystichum,[1] typified by the well-known *P. Lonchitis* constitutes a natural and well-defined group of ferns and has commonly had general recognition, recently even by American writers.[2] Its species are characterized, briefly, by their rigidity and erect habit, smooth firm dryish texture, mainly auriculate and spinulose or mucronate divisions, abundant chaff, free venation, and ordinarily by the orbicular centrally peltate indusia. The genus was divided by JOHN SMITH into two sections: the one, typified by *P. aculeatum* and *P. Lonchitis*, with fasciculate generally erect acaulose vernation; the other, typified by *P. coriaceum*, with uniserial sarmentose vernation. In view of that writer's natural bent for generic segregation along the very lines on which the species of this genus were placed in two sections, it would not have been surprising if he had accorded to each section generic rank. That such a disposition—in view of the two radically different types of vernation—was not made is significant, for it emphasizes SMITH's belief that in other respects the species are in too close agreement to warrant their division into two genera. There is, however, variation in one other particular; in certain species of both groups the indusium is caducous, or, as in *P. tenue*,[3] apparently quite wanting; but even in these cases the appearance of the plants is so unmistakably that of Polystichum and their agreement in the main particulars so essential that a rational course seems to necessitate their recognition as true members of the genus. We have thus a group of species which, though offering

[1] ROTH, Teut. Fl. Germ. **3**:69. 1800.

[2] GILBERT, in papers presented at the Boston Meeting (1898), 22. 1899. UNDERWOOD, Our native ferns, ed. 6, 115. 1900. DAVENPORT, Rhodora **4**:9. 1902, etc.

[3] GILBERT, Fern Bull. **8**:63. 1900.

variation in two lines, is nevertheless, in the characters enumerated at the beginning of this paper, one of the most unified to be found among the ferns. Indeed, as GILBERT has remarked, the general characters of the genus are so distinctive and obvious that the descriptive term *polystichoid* has come into rather common use.

The historic treatment of Polystichum, although of much interest, need not be here discussed at length. The genus has been made often to include diverse groups not properly associable with its typical members; and in several cases it has been an author readiest to admit numerous small genera who has failed to realize the essential unity of the group and to refuse to admit unlike forms. Thus MOORE retained *Cyclopeltis* J. Sm. under Polystichum, though it constitutes a most distinct natural genus. And more recently, DIELS,[4] while properly removing Cyclopeltis, nevertheless allows Phanerophlebia, Cyrtomium, and Adenoderris to remain within the genus. Cyrtomium and Phanerophlebia have recently been held distinct from each other and from Polystichum by UNDERWOOD,[5] and it now appears certain that Adenoderris, long ago founded by JOHN SMITH upon the anomalous *Aspidium glandulosum* of Hooker and Greville, represents a perfectly valid genus. The discovery of a second species of Adenoderris with very different venation, from Guatemala, is of interest and has seemed to render desirable the present notice of the genus and its relationship.

ADENODERRIS J. Sm. Hist. Ferns 222. 1875.[6]—Small plants of lax habit, distinct from Polystichum by their herbaceous texture, aspinulose margins, and dense glandular-pilose covering.—Type, *Aspidium glandulosum* Hook. and Grev. from Jamaica. Species two.

Adenoderris viscidula (Mett.).—*Aspidium glandulosum* Hook. and Grev. Icon. Fil. **2**: *pl. 140.* 1831; not *Aspidium glandulosum* Blume, Enum. Pl. Javae **2**: 144. 1828. *Adenoderris glandulosa* J. Sm. Hist. Ferns 223. 1875. *Aspidium viscidulum* Mett. Abhand. Senck. Nat. Gesells. **2**: 322. 1858.—The species was very fully characterized upon specimens received from several collectors in Jamaica and is the subject of an excellent illustration by HOOKER and GREVILLE. Lately it has been well described by JENMAN.[7] SMITH, basing his genus upon the single species, published the

[4] DIELS, in Engler and Prantl, Die natürlichen Pflanzenfamilien **1**[4]: 183–189. 1899.

[5] UNDERWOOD, Bull. Torr. Bot. Club **29**: 121–136. 1902. See also UNDERWOOD in Bull. Torr. Bot. Club **26**: 205–216. *pls. 359–360.* 1899.

[6] The genus seems to be published here for the first time, although the author cites the date 1852.

[7] Bull. Bot. Dept. Jamaica **2**: 197–198. 1895.

following characters: "Vernation fasciculate, erect, acaulose. Fronds 6 to 8 inches in length, oblong, lanceolate, pinnatifid, densely covered with pilose glands, decurrently attenuated to a short stipe. Veins pinnately forked. Receptacles punctiform, medial. Sori round. Indusium orbicular, occasionally reniform."[8] In habit the plant was said to be "totally at variance" with any species of Polystichum, and this opinion was formed entirely from herbarium specimens. An examination of material in the field indicates even more plainly how inappropriate has been the usual systematic association of this peculiar plant with true members of Polystichum. The fresh plants are spongy, very lax, and intensely viscid, and except for the peltate indusia have nothing to suggest a close relationship with the stiff smooth spinulose Polystichums. The venation of *A. viscidula*, which was not indicated by HOOKER and GREVILLE, is shown in *fig. 1*, which represents the middle portion of a Jamaican specimen (*Clute* no. 333; U. S. National Herbarium, no. 349588). This feature and the position of the sori are discussed under the next species.

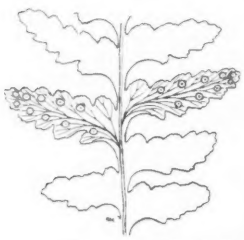

FIG. 1.—*Adenoderris viscidula* (Mett.) Maxon; natural size.

A. viscidula is known only from Jamaica and Cuba. JENMAN states that in Jamaica it occurs upon "rocky banks and skirts of forests 1500–3000 ft altitude; plentiful in one place at least between Gordontown and Guava Ridge. There is, however, but one sheet in the Jenman Herbarium at New York. Other Jamaican specimens are: *Clute* no. 333, collected above Gordontown, March 12, 1900, at an altitude of 450m; *Underwood* no. 2498, collected near the Green River (below Cinchona), April 22, 1903, at an altitude of 750m; and specimens collected by *D. E. Watt* at or near the last locality in May 1903. The Cuban record[9] is based upon *C. Wright* no. 1052. Specimens of this number in the D. C. Eaton herbarium are identical with the Jamaican plant; to them is attached Wright's original label stating that they were collected in rocky ravines on mountain sides near Josephine, October 25 (1859).

Adenoderris sororia, sp. nov.—A delicate plant of small size, the fronds glandular throughout. Rhizome slight, erect, having long fibrous rootlets rather thickly clothed with delicate bright brown chaff: fronds 8cm long, short-stipitate, spreading, oblong-lanceolate, deeply pinnatifid, with about

[8] Only orbicular indusia have been observed by the writer.

[9] Hooker, Sp. Fil. **4**:6. 1842.

ten pairs of usually subopposite to alternate pinnae; pinnae ovate to deltoid, the middle ones 10–13^{mm} long, the lowermost scarcely reduced, all deeply divided into about three blunt lobes upon both upper and lower margins, each lobe usually once soriferous near the outer side at the base, the sorus being borne at the *extremity* of a spur given off from the otherwise usually simple single veinlet; venation terminating well within the lobe; indusium minutely glandular, orbicular, centrally peltate.

FIG. 2.—*Adenoderris sororia* Maxon, n. sp.; natural size.

Founded upon no. 868 of *John Donnell Smith's* Guatemalan plants; said to have been collected by *von Türckheim* at Sesisp, Department of Alta Vera Paz, altitude 1200^{m}, March 1886, and distributed as *Aspidium glandulosum*. The most perfect material of this number the writer has seen is that preserved in the D. C. Eaton herbarium at Yale University, and this, having served for the accompanying illustration (*fig. 2*), may stand as the type, though the specimens in the United States National Herbarium and the herbarium of the New York Botanical Garden are of the same collection. Captain SMITH has stated (in litt.) that duplicates were presented also to the W. M. Canby, Philadelphia Academy, Kew, Berlin, Paris, and DeCandolle herbaria.

Adenoderris sororia is distinct in all states from *A. viscidula*, though in its lax habit, slight texture, aspinulose margins, and glandular covering it shows an undoubted generic alliance with that species. It differs specifically in its less size, bipinnatifid condition throughout (*A. viscidula* though larger is only deeply once-pinnatifid), more sparse glandular covering, and in its spreading simpler *included* venation, the sori being borne terminally at the apices of the veinlets. In *A. viscidula* the veinlets are pinnately forked and excurrent to the suberose margins, the sori being borne *dorsally, i. e.*, upon the veinlets and at some distance from the margin. These differences, while very marked, appear to be no more than specific, and SMITH's original generic diagnosis quoted above must therefore be amended as regards venation.

Both drawings, which are by Mr. H. D. HOUSE, are natural size. That of *A. sororia* represents parts of the third, fourth, fifth, sixth, and seventh pairs of pinnae of a frond of the type specimen.—WILLIAM R. MAXON.

CURRENT LITERATURE.

BOOK REVIEWS.

Rusts of Switzerland.

THREE NOTABLE WORKS on plant rusts have appeared within a year: KLEBAHN'S *Wirthswechselnde Rostpilze*, the first volume of SYDOW'S *Monographia Uredinearum*, and FISCHER'S *Die Uredineen der Schweiz*. Each of these works covers a distinct field, and in its own way marks advance in the elucidation of the world's rust flora, and in an understanding of the problems connected therewith. The last named work,[1] although embracing a limited region, is conceived upon such a broad plan, and carried out with so much completeness, that it serves as the best model yet produced for a uredineous manual.

A large number of the species found in Switzerland are cosmopolitan. Every species is described in detail, and with few unavoidable exceptions direct from specimens, and is also illustrated with outline drawings. The descriptions are especially full, embracing not only the usual characters, but those derived from the pycnidia, the peridial cells, and the germ pores. The illustrations are drawn to a uniform scale, and are skilfully made. Often a dozen or more teleutospores are shown. Usually uredospores are included, and always drawn in a normally upright position, a most commendable innovation. In most cases a transverse section of two or more peridial cells shows their varying thickness of wall and sculpturing, another helpful innovation.

The notes which follow the diagnoses, briefly stating how much is known of the life history of the species, are helpful and suggestive. The list of Swiss stations for each species is more especially of local value to collectors.

The systematic arrangement is essentially that of DIETEL in ENGLER and PRANTL'S *Pflanzenfamilien*. Under the genera the species are distributed by hosts and morphological characters, with a view to showing relationship. A general key on the same basis is provided, together with excellent indexes, and a full modern bibliography.

Three chapters are of unusual interest to the general mycologist. In one the distribution of the Uredineae in Switzerland is analyzed and discussed, taking into account the ecological factors controlling the hosts. In another the general classification and the grouping of species within the genera are considered in the light of the most probable hypotheses regarding the phylogenetic descent of the rusts. And in the third the difficult questions regarding the value of various morphological and biological characters for discrimination of species are presented.

[1] FISCHER, ED., Die Uredineen der Schweiz. Beitr. z. Kryptogamenflora der Schweiz. II[2] imp. 8vo. pp. xciv+590. *figs.* 342. Bern: J. K. Wyss. 1904. 20 *francs.*

This work by Dr. FISCHER, for which he has been preparing for more than a dozen years, is replete with new matter of great value and is altogether admirable. —J. C. ARTHUR.

MINOR NOTICES.

THE FOSSIL FRUITS from the lignites of Brandon, Vermont, are the subject of an important contribution by PERKINS.[2] In 1861 LESQUEREUX described twenty-one species from this locality, which he regarded as of approximately the same age as the Œningen stage of the Swiss Miocene. In 1902 KNOWLTON published a brief paper of forms from this locality which represents all we knew of this interesting flora up to the time of publication of the present paper. The deposits have been much obscured and inaccessible for a half century, until the coal famine of 1902, when the lignite came into demand locally as a substitute for coal. The state geologist, GEORGE H. PERKINS, was enabled to secure a magnificent collection of the fossil fruits during the mining operations, and the present paper contains the result of preliminary study of these collections. One hundred and eighteen species are recorded, and many new forms of more or less doubtful botanical affinities are described. These are largely included in the following new genera: *Monocarpellites* (11 spp.), *Hicoroides* (5), *Bicarpellites* (5), *Brandonia*, *Rubioides*, *Sapindoides* (6), and *Prunoides*. The illustrations consist chiefly of photographs of type specimens, which are perhaps less satisfactory in showing details than careful drawings. The flora is unique in the abundance and variety of its fruits, and it is to be hoped that future study by Dr. PERKINS will demonstrate with more precision the exact age of the formation containing them. The accompanying clays should be searched for leaf remains.—EDWARD W. BERRY.

POPULAR ACCOUNTS of soil inoculation for legumes devised by MOORE have attracted wide attention, so that it is of special interest to receive his own account.[3] The nitrogen is fixed by the tubercle-forming bacteria within their bodies. This was determined by cultures in flasks containing nutrient solutions without nitrogen. There was no increase of nitrogen in the solution, but a marked increase in the organisms themselves. In its biology the organism is therefore considered a parasite. Later the plant is able to overcome the parasite and profit by the nitrogen which has been fixed. When grown on nitrogenous media, it was found that the organism lost both its power of infecting leguminous plants and its power of fixing nitrogen. In non-nitrogenous media both of these properties were retained. The failure of NOBBES's attempts in Germany a few years ago to put upon the market pure cultures of this organism can probably be attributed to lack of recognition of this fact. As a result of these studies MOORE has devised

[2] PERKINS, GEO. H., Description of species found in the Tertiary Lignite of Brandon, Vermont. Rept. State Geologist, Vt. 1903–1904. pp. 174–212. *pls. 75–81.* 1904.

[3] MOORE, G. T., Soil inoculation for legumes, etc. U. S. Dept. of Agric., Bureau of Pl. Industry, Bull. 71, pp. 72. *pls. 10.* 1905.

a method of putting up for distribution pure cultures of *Pseudomonas radicicola*, grown in nitrogen-free media and dried on cotton immersed in the culture. These cultures are sent out by the U. S. Department of Agriculture together with packages of nutrient salts to multiply the organism. The mass-culture thus obtained is used to inoculate the seed or the soil. Numerous reports from farmers of all states indicate that this method will prove successful and practicable.—H. HASSELBRING.

THE OFFICE of Experiment Stations of the U. S. Department of Agriculture has recently issued a valuable syllabus[4] of the diseases of the Irish potato in the United States by STEWART and EUSTACE of the New York (Geneva) Experiment Station. This is the second of a new series of publications designed primarily to assist farmers' institute lecturers in the presentation of various important subjects to farmers. Each lecture is accompanied by a set of lantern slides, there being in this case forty-seven slides in the set. Arrangements have been made to loan these slides to lecturers, or they may be purchased through the Department of Agriculture at a very moderate cost. Both the syllabus and the accompanying set of slides should prove of great value to teachers of plant pathology in presenting the diseases of the potato. A brief bibliography is appended to the syllabus, which contains references to the most important American literature of this subject.—E. MEAD WILLCOX.

AN UNDATED EDITION of ANDREWS'S *Botany all the year round* has been issued, which differs from the preceding one[5] only in being bound into one volume with a flora by W. NEVIN GEDDES.[6] This flora brings together compact and simple descriptions of the more common flowering plants, not only native and naturalized, but also cultivated, and includes about 1,250 species. The sequence of families is an interesting reminiscence of the old time classification, and it is quite a pleasure to see gymnosperms once more as a subclass of "Exogens or Dicotyledons." The nomenclature, too, is frankly "conservative and eclectic;" but the book does as well as any other to enable a student to find names for plants, which seems to be regarded as a very valuable exercise.—J. M. C.

THE FOURTH PART of SARGENT'S *Trees and shrubs*[7] contains plates and descriptive text of species of Acer (13), Parthenocissus (3), Malus, Oroxylum, Phellodendron (3), Arctostaphylos (2), Dracaena, and Pinus (2). There are

[4] STEWART, F. C. and EUSTACE, H. J., Syllabus of illustrated lecture on potato diseases and their treatment. Office of Exp. Stat. U. S. Dept. Agr. Farmers' Inst. Lecture **2**:1–30. 1904.

[5] See BOT. GAZETTE **35**:439. 1903.

[6] GEDDES, W. NEVIN, A brief flora of the eastern United States. New York, Cincinnati, Chicago: American Book Company, with no date.

[7] SARGENT, C. S., Trees and shrubs. Illustrations of new or little known ligneous plants, prepared chiefly from material at the Arnold Arboretum of Harvard University. Part IV. pp. 151–217. *pls. 76–100*. Boston and New York: Houghton, Mifflin & Company. 1905. $5.00.

two new Chinese maples, a conspectus of the maples of eastern continental Asia, two new American species of Parthenocissus, and one new Chinese Oroxylum, by ALFRED REHDER; a new Phellodendron, by CHARLES S. SARGENT; two new Californian species of Arctostaphylos, by ALICE EASTWOOD; a new Central American Dracaena, by JOHN DONNELL-SMITH; and two new Mexican pines by G. R. SHAW. This part closes the first volume, and contains the title-page, index, and corrections.—J. M. C.

HERBERT STONE[8] has brought together a most interesting collection of descriptions and excellent photomicrographs of commercial timbers. Being able to draw upon the British colonies for material, the list is unusually full. An introductory chapter gives a general account of the varying structure of wood from the standpoint of one who has a practical acquaintance with timber; and this is followed by practical hints as to its identification. The list of timbers includes 247 numbers, each species with a very full description, all the genera being illustrated by photomicrographs.—J. M. C.

THREE PARTS of an illustrated handbook of the angiospermous woody plants native or hardy in middle Europe by C. K. SCHNEIDER have appeared.[9] The illustrations are numerous and excellent, floral dissections, fruits, and leaves being given, and in many cases fine half tones of characteristic bark. The ENGLER and PRANTL sequence is used. The first part includes Salicacae to Fagaceae, with 95 illustrations; the second part, Fagaceae (Quercus) to Berberidaceae, with 102 illustrations; the third part, Berberidaceae to Spiraceae, with 90 illustrations.—J. M. C.

A SECOND EDITION of GROUT'S *Mosses with a hand lens* has been published,[10] including the common liverworts. The review of the first edition[11] stated the general scope and purpose of the book. The demand for a new edition has justified the undertaking, and with the addition of much new material the author believes that he has reached the limit of the hand lens. The addition of the common liverworts represents a very natural extension of the author's method, and introduces beginners to a group they must have encountered repeatedly in collecting mosses.—J. M. C.

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[8] STONE, HERBERT, The timbers of commerce and their identification. Illustrated with 186 photomicrographs by Arthur Deane. Imp. 8vo. pp. xxxviii+311. London: William Rider & Son. 1904.

[9] SCHNEIDER, CAMILLO KARL, Illustriertes Handbuch der Laubholzkunde. Charakteristik der in Mitteleuropa heimischen und im Freien angepflanzten angiospermem Geholz-Arten und Formen mit Ausschluss der Bambuseen und Kakteen. Jena: Gustav Fischer. 1904. Each part *M*4.

[10] GROUT, A. J., Mosses with a hand lens. A non-technical handbook of the more common and more easily recognized mosses of the northeastern United States. Illustrated by MARY V. THAYER. Second edition, revised, enlarged, and including the hepatics. 8vo. pp. xvi+208. *pls. 39. figs. 118.* New York: The Author, 360 Lenox Road, Flatbush. 1905.

[11] BOT. GAZETTE **31**:132. 1900.

NUMBERS 221 and 222 of ENGLER and PRANTL'S *Pflanzenfamilien* have appeared, the former containing a continuation of the lichens by A. ZAHLBRUCKNER, the latter a continuation of the mosses by V. F. BROTHERUS.—J. M. C.

THE SEVENTH FASCICLE of DALLA TORRE and HARMS'S *Genera Siphonogamarum*[12] has just appeared, including genera from Acanthaceae (7938. Echinacanthus) to Compositae (9371. Liabum).—J. M. C.

NOTES FOR STUDENTS.

PALEOBOTANICAL NOTES.—BRABENEC[13] records thirteen species of plants from a new locality, Holedeč, in the Saaz basin of northern Bohemia, describing new species in Carya, Acacia, Paliurus, and Porana.—BERRY[14] describes additional material from the Cretaceous near Cliffwood, N. J., consisting of rather fragmentary remains of nine additions to that flora, making the total number of known forms ninety-five.—The same author describes[15] a new palm, represented by abundant but fragmentary remains of leaves, from the mid-Cretaceous of the coastal plain in Delaware and Maryland.—In notes on Tertiary plants PENHALLOW[16] describes wood of Taxodium and Thuja from the basal Eocene of Alberta, and of Cupressoxylon from the Cretaceous of Assiniboia. Wood of *Pseudotsuga Douglasii* is identified from near Bozeman, Montana, and the characteristic Pleistocene *Larix americana* (*Larix laricina*) is identified from Georgia. The latter find is most interesting, showing as it does that the larch had pushed about 480 miles farther south than its present southern limit during the Scarborough period of the Glacial. Wood of *Pinus rigida* is recorded from the Pleistocene of New York; and *Juniperus californica* and *Pseudotsuga macrocarpa* are recorded from the Pleistocene at Orleans, Humboldt county, California. The paper concludes with an enumeration of a number of species, based on leaves and wood, from the well-known Don River Pleistocene of Canada.—WHITE[17] has a paper on "Fossil plants of the group Cycadofilices," in which the chief points of interest in this most interesting group are enumerated. He concludes, with WILLIAMSON, that secondary wood originated, polyphyletically and polychronously, as an engineering feature. It is suggested that the heterosporous filicean ancestors of the group Pteridospermae will be found in the lowest Carboniferous if not in the Upper Devonian. Should the author's *Kalymma grandis* prove to

[12] DALLA TORRE, C. G. DE, and HARMS, H., Genera Siphonogamarum ad systema Englerianum conscripta. Fasc. 7. pp. 481–560. Leipzig: Wilhelm Engelmann. 1905. *M6.*

[13] BRABENEC, F., Ueber einen neuen fundort v. Tertiären Pfl., etc. v. Saazer Schichten Bull. Inter. Acad. Sci. Bohême, 1904. pp. 8 *pl. 1.*

[14] BERRY, E. W., Bull. Torr. Bot. Club **32**:43–48. *pls. 1–2.* 1905.

[15] BERRY, E. W., Torreya **5**:30–33. *figs. 2.* 1905.

[16] PENHALLOW, D. P., Trans. Roy. Soc. Can. II. **10**:57–76. 1904.

[17] WHITE, DAVID, U. S. Geol. Surv. Professional Paper No. 35: pp. 35–84. *pls. 2–6.* 1905.

be pteridospermic, as is expected, it will serve to emphasize the evidence of the fossil wood (Dadoxylon) as to the occurrence of gymnospermous-like plants in the middle Devonian (Genesee), a time nearly as remote as that furnishing the oldest known ferns of undoubted authenticity, and would seem to render the search for ancestral forms of these comparatively highly organized pteridospermic types futile.—The same author gives[18] a systematic account of the Devonian flora of the Perry basin, Maine, listing twenty-nine species, not including nine more or less doubtful species which DAWSON reported from this locality. Several new generic and specific types are described, and numerous nomenclatorial points are cleared up. The paper is fully illustrated.—EDWARD W. BERRY.

CLAUSSEN[19] gives an account of the life history of the ascomycete Boudiera. The sexual organs consist of short filaments which are developed in clusters and become very much twisted together, presenting spirally wound structures somewhat resembling the sexual organs of Gymnoascus. These filaments are differentiated into antheridia and ascogonia which fuse. After fertilization a cluster of short ascogenic hyphae arise that develop a small group of asci which become loosely enveloped by surrounding paraphyses from sterile portions of the mycelium. The female organ is the thicker filament of the two and consists of an ascogonium, containing several nuclei, which bears a terminal sterile cell, perhaps two. The nuclei in the sterile cell break down, and it finally becomes the medium (conjugation tube or trichogyne) through which the protoplasm from the antheridium enters the ascogonium. The antheridial filament is thinner and also contains several nuclei, which after the fusion with the sterile terminal cell pass into the ascogonium. The fertilized ascogonium then contains some ten or twelve nuclei and the antheridial filament and sterile terminal cell are empty. The nuclei in the fertilized ascogonium fuse in pairs. The empty antheridial filament and sterile cell collapse. The fertilized ascogonium increases in size, the fusion nuclei become conspicuous, and it finally divides into several cells by cross walls. The ascogenic hyphae are very short and the tips take at once the form of a hook and contain four nuclei. One nucleus lies in the tip, another in the stalk, and the remaining two in the bent portion of the hook. The four-nucleate condition is derived from a two-nucleate, but the preceding history is not known. The pair of nuclei in the bent portion of the hook become separated by walls from the tip and base of the filament and shortly coalesce; this cell becoming then a young ascus containing the single fusion nucleus. Thus each ascogenic hypha develops a single ascus. The terminal cell remains indefinitely, but there is no development from it. Several asci are formed from one ascogonium, but not simultaneously. Their number is believed to agree with the number of fusion nuclei in the ascogonium. The process of spore formation appears to follow HARPER'S account, but

[18] WHITE, DAVID, Smithsonian Miscellaneous Coll. **47**: 377–390. *pls. 53–55.* 1905.

[19] CLAUSSEN, P., Zur Entwickelungsgeschichte der Ascomyceten Boudiera. Bot. Zeit. **63**[1]: 1–28. *pls. 1–3.* 1905.

details are not given. The paper ends with a long historical discussion of the disputed sexuality of the ascomycetes and supports HARPER'S conclusions.—B. M. DAVIS.

ITEMS OF TAXONOMIC INTEREST are as follows: T. MAKINO (Bot. Mag. Tokyo **18**:156. 1904) has separated the Japanese form referred to *Croomia pauciflora* T. & G. as a distinct new species (*C. kiusiana*).—W. B. HEMSLEY (Hooker's Icon. Plant. IV. **8**: *pl. 2781.* 1905) has named the type of a new West Australian genus of Compositae (Helichryseae) *Thiseltonia Dyeri*.—PH. VAN TIEGHEM (Jour. Botanique **19**:45–58. 1905) has made the genus Octocnema (provisionally placed under Olacaceae) the type of a distinct family (*Octocnemaceae*).—A. A. HELLER (Muhlenbergia **1**:111–118. 1905) has described new Californian species of Ribes, Trifolium, and Stachys.—J. CARDOT (Bull. Herb. Boiss. **5**:208. 1905) has described a new genus (*Alophosia*) of mosses from San Miguel.—L. RADLKOFER (*idem* 222) has described a new genus (*Sisyrolepis*) of Sapindaceae from Siam.—CARL MEZ (*idem* 232) has described a new Venezuelan genus (*Glomeropitcairnia*) of Bromeliaceae with two species.—J. C. ARTHUR (Annales Mycol. **3**:18. 1905) has described a new genus (*Bacodromus*) of Uredineae with two species.—E. L. GREENE (Ottawa Nat. **18**:215–216. 1905) has described new Canadian species of Malus and Fragaria (2).—W. FAWCETT and A. B. RENDLE (Trans. Linn. Soc. London II Bot. **7**:1–13. *pls. 1–2.* 1904), in a monograph of the Jamaican species of Lepanthes (Orchidaceae), recognize 12 species, describing 5 as new.—N. L. BRITTON (Bull. N. Y. Bot. Garden **3**:441–453. 1905), in his first "Contributions to the flora of the Bahama Islands," has published 15 new species and a new genus (*Bracea*) of Apocynaceae.—J. J. SMITH (Recueil Trav. Bot. Neerl. **1**:49–51. *pl. 2.* 1904) has described a new genus (*Gynoglottis*) of Orchidaceae from Sumatra.—M. L. FERNALD (Rhodora **7**:47–49. 1905) has described a new species of Comandra that ranges from Quebec to Assiniboia and Kansas.—F. STEPHANI (Hedwigia **44**:74. 1905) has described two new Himalayan genera (*Gollaniella* and *Massalongoa*) of Hepaticae.—L. DIELS and E. PRITZEL (Bot. Jahrb. **35**:564. 1905) have published a new Australian genus (*Pentaptilon*) of Goodeniaceae.—G. E. DAVENPORT (Fern Bulletin **13**:18–21. 1905) has published a new Mexican Aneimia, with plates.—A. and E. S. GEPP (Jour. Botany **43**:108. *pl. 470.* 1905) have described a new genus (*Leptosarca*) of the red algae from the South Orkneys.—J. M. C.

BERGH'S second paper[20] on the formation of heterotypic chromosomes deals with the behavior of the chromatin from the telophase of the last division in the sporogenous tissue up to the strepsinema stage of the first mitosis in the microspore mother-cell. In a very early prophase of the first mitosis in the mother-cell, slender filaments are formed from the chromosomes of the sporogenous cell. Synapsis then takes place and at its close the filaments appear as the

[20] BERGHS, JULES, La formation des chromosomes hétérotypiques dans la sporogénèse végétale. II. Depuis la sporogonie jusqu'au spirème définitif dans la microsporogénèse de l'*Allium fistulosum*. La Cellule **21**:383–397. *pl. 1.* 1904.

"thick spirem." During synapsis the dual nature of the filaments is evident. It is due to a longitudinal approximation of two filaments and leads to the formation of the thick spirem. The so-called longitudinal division is merely the reappearance of the line of approximation. Consequently the reduction in the number of chromosomes seen in the prophase of the heterotypic division is only an apparent reduction, due to the approximation of two somatic chromosomes, which in the mature chromosomes play the rôle of daughter chromosomes. The reduction is really accomplished by the first mitosis, which separates these daughter chromosomes.

The third paper[21] in the series deals with microsporogenesis in *Convallaria majalis*. The results confirm the conclusions already drawn from the author's study of *Allium fistulosum* and *Lilium speciosum*. The two daughter chromosomes which constitute the mature heterotypic chromosome represent two longitudinal parts of one segment of the thick spirem. This thick spirem is formed by the longitudinal approximation of slender chromatic filaments, two by two. This approximation takes place during the synaptic contraction, and the separation takes place in the strepsinema stage, resulting in an apparent longitudinal division. The heterotypic division is a reduction division, which distributes to the two poles complete somatic chromosomes.—C. J. CHAMBERLAIN.

THE RESEARCHES of SCHWARZ, ELFVING, VOCHTING, RACIBORSKI, and others on the influence of gravitation upon the rate of growth of erect and inverted organs have been extended by the investigations of HERING[22] carried on in PFEFFER'S laboratory. This work adds support to the thesis generally maintained by earlier authors, that while the rate of growth of geotropic organs in natural position (vertical) is not affected by gravitation, such organs when inverted manifest a checking of the rate of growth in length. Improvement upon ELFVING'S technique was secured by using artificial light of constant intensity instead of sunlight for keeping the organs parallel with gravitation; also by substituting a non-shrivelling substratum for bread crumbs for the growing of fungi. For such organs as could not be maintained in a vertical position by the use of light, recourse was taken to the use of pulleys and weights. The experimental material employed includes both positively and negatively geotropic organs as found in members of various genera of fungi, of monocotyledons, and of dicotyledons, as well as the branches of weeping trees. While nodes of grasses constitute an exception, the uniformity of behavior revealed by numerous other forms is so great as to permit a formulation of this general statement, namely, parallelotropic organs as a consequence of inversion suffer a reduction in the rate of growth in length. In the case of the sporangiophores of Phycomyces prolonged inversion is followed by

[21] BERGHS, JULES, La formation des chromosomes hétérotypiques dans la sporogénèse végétale. III. La microsporogénèse de *Convallaria majalis*. La Cellule **22**:41–53. *pl. 1.* 1904.

[22] HERING, GEORG, Untersuchungen über das Wachsthum inversgesteller Pflanzenorgane. Jahrb. Wiss. Bot. **40**:499–562. 1904.

accelerated growth, although a temporary inversion (one hour) is followed by diminished growth. In the case of the descending branches of weeping trees, gravitation not only causes a reduced rate of growth, but also determines the locus of origin of new shoots. Growth correlations similar to those observed by RACIBORSKI in tropical lianas were obtained.—RAYMOND H. POND.

LAWRENCE[23] has published a bulletin containing a summary of our present knowledge and the results of his own investigations of a serious apple disease found thus far only in Oregon and Washington, that was first described by CORDLEY[24] under the common name "apple tree anthracnose." The disease is also known under the following common names: Oregon canker, black canker, blackspot apple canker, blackspot, deadspot, and sour sap disease. The present writer proposes that the disease be known as the "blackspot canker." The fungus causing it was described by CORDLEY[25] under the name *Gloeosporium malicorticis*, and has been described also by PECK[26] under the name *Macrophoma curvispora*. This fungus is known to injure apple trees seriously in the Pacific northwest by the production of cankers upon the smaller twigs and branches. Similar cankers were produced by inoculation from apple cankers upon the cherry, plum, prune, and peach by LAWRENCE, and he is inclined to the belief that the same fungus is responsible for cankers upon all of the hosts named. As a rule cankers do not appear upon the larger branches and the trunk, owing apparently to the resistance offered by the corky outer layers of the bark of such regions to the penetration of the fungus. LAWRENCE here reports for the first time that the same fungus causes also a dry rot of stored apples that may be rather serious under certain conditions. The cankers may appear within a week from the time of infection; ordinarily appearing from November to February, rapidly completing their development after renewal of growth on the part of the host, and becoming mature by the next June or July. No definite statement can yet be made regarding either treatment of the disease or as to possible resistant varieties.—E. MEAD WILCOX.

A MUCH NEEDED INVESTIGATION of the rôle of latex in the metabolism of plants has been commenced by KNIEP,[27] and if continued may lead to valuable results. The paper here cited is presented in four sections. The introduction carefully reviews the literature, showing that theories are not supported by facts and that experimental results do not agree. The second section contains experi-

[23] LAWRENCE, W. H., Blackspot canker. Bull. Wash. Exp. Sta. 66. pp. 35. *pls. 13*. 1904.

[24] CORDLEY, A. B., Apple tree anthracnose: a new fungus disease. Bull. Oregon Exp. Sta. 60. pp. 3–8. *pls. 1–3*. 1900.

[25] CORDLEY, A. B., Some observations on apple tree anthracnose. BOT. GAZETTE **30**:48–58. *figs. 1–12*. 1900.

[26] PECK, C. H., New species of fungus. Bull. Torr. Bot. Club **27**:21. 1900.

[27] KNIEP, HANS, Ueber die Bedeutung des Milchsafts der Pflanzen. Flora **94**:129–205. 1905.

mental evidence obtained by the author, which establishes that the latex partici pates very little, if at all, in the translocation of nutrient substances. Seedlings subjected to conditions intended to force an exhaustion of storage products showed no difference as to quantity or form of starch grains in the latex as compared with controls. The author does not regard the latex starch of the euphorbias with which he experimented as a typical storage product. SCHIMPER's results that the latex tubes of the euphorbias do not participate in the depletion of carbohydrates is verified. The third section comprises an anatomical study of thirty-six species distributed in seven families of laticiferous plants. DEBARY's view that latex tubes and sieve tubes are reciprocal tissues is rejected. The opinion of HABERLANDT that latex tubes function as translocation parenchyma is also discredited. The final section presents an ecological view of the problem, and two functions are assigned as ecological, namely, the closing of wounds by the exudation of latex, and protection against animals secured by presence of poisonous, acrid, or ill-smelling substances. That the problem remains unsolved, and that the chief value of his paper consists in formulating the present status of investigation as well as in indicating promising lines of research, is fully realized by the author.—RAYMOND H. POND.

MITOSIS in the salamander (*Salamandra maculata*) has been investigated by KOWALSKI[28] under the direction of GREGOIRE. The object of the investigation was to compare typical animal mitoses with the mitoses in the roots of Trillium as described by GREGOIRE and WYGAERTS (see BOT. GAZETTE **38**:396. 1904). Mitosis was studied in various tissues, but principally in the epithelial cells. In general it may be said that even in minute details the structures and the sequence of events are essentially the same as in Trillium. In the telophase the action of the nuclear sap outside the chromosome results in the production of anastomoses between the neighboring chromosomes and between parts of the same chromosome; while the action of the sap within the chromosome brings about an alveolar and reticular structure. Each chromosome forms a network of lamellae and filaments of various shapes and sizes which cross each other in every direction. The entire chromatic network of the nucleus is made up of the individual networks. The behavior of the chromatin in the prophases is strong evidence in favor of the individuality of the chromosome. The longitudinal splitting of the chromosome is not due to the bipartition of a series of chromatic disks imbedded in a substratum, but consists simply in the longitudinal cleavage of a chromatic ribbon. No disk spirum is formed at the telophase, nor is any spirem formed at the prophase. The nuclear membrane is formed by the condensation of the cytoplasm bordering upon the nuclear vacuole, and at its formation no cytoplasm is included within the nuclear cavity.—C. J. CHAMBERLAIN.

[28] KOWALSKI, J., Reconstitution du noyau et formation des chromosomes dans les cinèses somatiques de la larve de Salamandre. La Cellule **21**:349–377. *pls. 1–2* 1904.

SEVERAL RECENT INVESTIGATIONS have brought to light a curious condition in certain plankton diatoms which is believed to be a method of spore formation. The contents of certain cells break up into a number of small bodies which are termed "microspores." These structures have been recently investigated by KARSTEN[29] in a species of Corethron. Their development begins in this form with a remarkable increase in the number of nuclei in a single diatom, until as many as 128 are present. The nuclei divide mitotically and the protoplasm successively until this large number of bodies are found, which however often remain connected with one another by delicate fibrils of protoplasm. KARSTEN believes that "microspores" which escape from the mother diatom are gametes and unite with one another in pairs to form a zygospore. Further, there is evidence that the zygospore gives rise to two new diatoms and that each of these contains at first two nuclei, one of which afterwards breaks down. If this should be established, we should have presented in the diatoms a method of sexual reproduction followed by a type of germination with two successive mitoses apparently of the same character as in the desmids. It is fair to say, however, that KARSTEN seems to draw his conclusions from a rather imperfect series of stages.—B. M. DAVIS.

PELETRISOT[30] has published an extended study of the development of the seeds of Ericaceae, including in his examination twenty-six genera and sixty-two species. Such a survey can only mean a glimpse of a general situation, which subsequent research will develop properly. Aside from anatomical details of tissues, which can hardly be related as yet, the general morphological situation seems to be about as follows. The most striking feature of the embryo sac is the development of a large haustorial pouch at each end. This haustorium varies much in size in different genera, reaching its maximum in Arbutus; and also in its degree of separation from the central cavity, sometimes being separated only by a slight constriction, and in other cases by a narrow, more or less prolonged neck. In many of the genera there is also a well-developed jacket-layer investing the central cavity of the sac. The synergids are often beaked and extended into the micropyle, and the antipodals are ephemeral.

Aside from the extent of the survey and the prolix presentation, the most notable feature of the paper is the absence of all knowledge of any English or American publications dealing with similar haustorial developments.—J. M. C

THE POSITION of the monocotyledons is discussed in a recent paper by FRITSCH.[31] Since the geological history is so fragmentary, we must rely upon the evidence of comparative morphology. After discussing this evidence,

[29] KARSTEN, G., Die sogenannten "Mikrosporen" der Plankton-Diatomeen und ihre weitere Entwickelung beobachtet an *Corethron Valdiviae*. Ber. Deutsch. Bot. Gesells. **22**:544–554. *pl. 23*. 1904.

[30] PELETRISOT, C.-N., Développement et structure de la graine chez les Ericacées. Jour. Botanique **18**:309–367, 386–402. *figs. 173*. 1904.

[31] FRITSCH, K., Die Stellung der Monokotylen im Pflanzensystem. Engler's Bot. Jahrb. Beiblatt No. 79. pp. 22–40. 1905.

including recent investigations in anatomy, the conclusion is reached that the Ranales and Helobiales are related groups; not, however, that any particular family in one has come from a family in the other, but that both have been differentiated from a common ancestry. HALLIER's theory that the entire group of the angiosperms is monophyletic and that it has come from a Ranales-like type, is, according to FRITSCH, a broader conclusion than is warranted by the present condition of our knowledge. Since the dicotyledons show some characters more nearly resembling conditions found in gymnosperms, the dicotyledons should be placed before the monocotyledons in any scheme of classification.—C. J. CHAMBERLAIN.

SHIBATA[32] has investigated the chemotaxis of the sperms of Isoetes. He finds the stimulation limit to be $m/20000$ malic acid. Of seventy other substances tested in concentrations of $m/10$ to $M/100$, only neutral salts of succinic, fumaric, and *d*-tartaric acids, which are in structure closely allied to malic acid, were attractive, the lower limit being $m/100$ to $5m/1000$. WEBER's law was found applicable. The relative chemotactic effect of H-ions was found equal in various mineral acids and salts; similarly, OH-ions from equivalent solutions of alkalies acted alike. Other interesting results bear on negative chemotaxis of anions, osmotaxis, and the nature of the reactions. The notice is a preliminary one.—C. R. B.

KOERNICKE[33] has reviewed several recent cytological papers, thus bringing his work on "The present condition of cell studies" up to date. The only important change of opinion noted is in regard to the occurrence of a reduction division in plants. The evidence given in the previous paper was against such a division, but in light of the more recent researches, especially those of FARMER and MOORE, KOERNICKE believes that the evidence in favor of reduction division is gradually increasing. The work of WILLIAMS, GREGORY, LOTSY, ROSENBERG, BOVERI, GRÉGOIRE and WYGAERTS, and HÄCKER are considered in this summary.—CHARLES J. CHAMBERLAIN.

WIESNER,[34] who has already pointed out two cases of the dropping of leaves which he calls *Sommerlaubfall* and *Treiblaubfall*, adds another, due to high temperature combined with drouth, which he calls *Hitzelaubfall*. The death of the leaves is due to excessive transpiration not covered by the available water. Naturally the peripheral leaves and especially those reached most directly by the sun are most affected. Observations are adduced from the author's extended tour in the United States during the months of August and September.—C. R. B.

[32] SHIBATA, K., Studien über die Chemotaxis von Isoetes-Spermatozoiden. Ber. Deutsch. Bot. Gesells. **22**:478–484. 1904.

[33] KOERNICKE, M., Die neueren Arbeiten über die Chromosomreduktion im Pflanzenreich und daran anschleissende karyokinetische Probleme. Bot. Zeitung 62^1:305–314. 1904.

[34] WIESNER, J., Ueber den Hitzelaubfall. Ber. Deutsch. Bot. Gesells. **22**:501–505. 1904.

STEMS OF CALAMITES with attached roots are rare, but several specimens have been described by MASLEN,[35] whose account clears up several doubtful points. The roots in question are mainly adventitious and spring from the nodes of the aerial as well as the subterranean stems. These roots may be distinguished from branches among other ways by their point of origin, which however is different from that of the roots of Equisetum. The writer finds no evidence for connecting the roots with the infranodal organs described by WILLIAMSON.—M. A. CHRYSLER.

GUIGNARD[36] has presented further interesting details in connection with double fertilization in the Malvaceae, using *Althaea rosea* and *Hibiscus Trionum*. Each large and very characteristic pollen grain puts out several pollen tubes, which advance through the tissues of the style and the nucellus in a peculiar way. In Hibiscus, when the embryo sac is reached, the tube branches irregularly. The formation of the generative nucleus and male cells, and the occurrence of starch are described in detail.—J. M. C.

MISS SARGANT and Miss ROBERTSON[37] call attention to the fact that the furrows on the dorsal face of the scutellum in *Zea Mais* should be regarded as glands which increase the extent of secreting surface. These glands are situated in contact with the floury part of the endosperm, and so secure a rapid transfer of food to the seedling while it as yet has no assimilating organs. An interesting observation is the presence of amphivasal strands and of transfusion tissue in the scutellum.—M. A. CHRYSLER.

DRABBLE'S studies on the roots of palms[38] show that the polystelic condition found in Areca is not exceptional, but that it commonly occurs in the proximal region of secondary roots. These separate steles usually fuse in the distal region of the root to form a single cylinder; certain vascular strands, however, may remain unfused and constitute the well-known medullary strands. The monostelic central cylinder of palm roots has thus by no means a simple origin.—M. A. CHRYSLER.

WALLER finds[39] that practically no sea plants give blaze currents following an induction shock, while practically no land plants fail to do so. Resistance of young tissues is very high, and is lowered much by one shock, which

[35] MASLEN, A. J., The relation of root to stem in Calamites. Ann. Botany **19**:61–74. *pls. 1–2.* 1905.

[36] GUIGNARD, L., La double fécondation chez les Malvacées. Jour. Botanique **18**:296–308. *figs. 16.* 1904.

[37] SARGANT, E., and ROBERTSON, A., The anatomy of the scutellum in *Zea Mais* Ann. Botany **19**:115–124. *pl. 5.* 1905.

[38] DRABBLE, ERIC, On the anatomy of the roots of palms. Trans. Linn. Soc. London. II. Bot. **6**:427–490. *pls. 48–51.* 1904.

[39] WALLER, A. D., On the blaze currents of vegetable tissues. Jour. Linn. Soc. Bot. **37**:32–50. 1904.

probably means that the lacking electrolytes are produced by dissociation upon passing the induction current. Killed tissues show no blaze currents.—C. R. B.

TREBOUX[40] in a preliminary paper announces that for chlorophyllous plants the source of N *par excellence* is the ammonia radicle, rather than amido-acids. The significance whereof lies in its relation to a general theory of proteid synthesis to which the author has been led by his experiments.—C. R. B.

VON SPIESS shows[41] that the so-called green aleurone does not exist, such grains being really more or less degenerate chloroplasts. Bluish aleurone grains occur in maize, the color being due to anthocyan.—C. R. B.

GATIN[42] has described cases of polyembryony in two palms, *Phoenix canariensis* and *Pinanga patula*, but gives no account of the origin of the embryos.—J. M. C.

BONNIER[43] has compared in detail the embryo sac of gymnosperms and angiosperms, reaching the conclusion that the most fundamental difference between the two groups is that in gymnosperms all the eggs formed in an embryo sac are alike; while in angiosperms the two eggs usually produced are unlike, one forming the embryo, the development from the other stopping at the proembryo stage (endosperm). This is an effort to include double fertilization in contrasting gymnosperms and angiosperms, but does not explain anything.—J. M. C.

MISS RIDDLE[44] has studied the development of *Staphylea trijoliata* from the megasporangiate archesporial cell of the embryo. The account presents no unusual features, but it is a matter of interest to note that double fertilization was observed. A definite account of some of the forms of Sapindales was much needed.—J. M. C.

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[40] TREBOUX, O., Zur Stickstoff-Ernährung der grünen Pflanzen. Ber. Deutsch. Bot. Gesells. **22**:570–572. 1905.

[41] VON SPIESS, KARL, Ueber die Farbstoffe des Aleurone. Oesterr. Bot. Zeits. **54**:440–446. 1904.

[42] GATIN, C. L., Quelques cas de polyembryonie chez plusieurs espèces de palmiers. Rev. Gén. Botanique **17**:60–65. 1905.

[43] BONNIER, GASTON, Remarques sur la comparaison entre les angiospermes et les gymnospermes. Rev. Gén. Botanique **17**:97-108. *figs. 6.* 1905.

[44] RIDDLE LUMINA C., Development of the embryo sac and embryo of *Staphylea trijoliata*. Ohio Naturalist **5**: 320-325. *pls. 19-20.* 1905.

NEWS.

PROFESSOR F. W. OLIVER, University College, London, has been elected a Fellow of the Royal Society.

DR. E. PFITZER, University of Heidelberg, has been elected associate member of the Royal Botanical Society of Belgium.

ALFRED ERNST, professor of botany at Zürich, has received from the government $1000 for botanical studies at Buitenzorg.

DR. GEORGE BITTER, docent in botany at Münster, has been appointed director of the Botanical Garden at Bremen.—*Science*.

PROFESSOR HUGO DEVRIES, University of Amsterdam, has been elected associate member of the Royal Academy of Belgium.

W. A. KELLERMAN has returned from a three months' exploration of Guatemala with a large amount of material, especially parasitic fungi.

PROFESSOR E. C. JEFFREY, Harvard University, has received a grant of $200 from the Elizabeth Thompson Science Fund "for the study of cupressineous conifers."

A. S. HITCHCOCK, U. S. Department of Agriculture, has been transferred to the Office of Botanical Investigations and Experiments, his position being styled "systematic agrostologist."

PROFESSOR THEOPHILE DURAND, director of the Botanical Garden at Brussels, and Professor JEAN MASSART, University of Brussels, have been elected corresponding members of the Royal Academy of Belgium.

F. E. LLOYD, Teachers College, Columbia University, has received a grant of $500 from the Carnegie Institution to aid him in continuing his studies on stomatal action and transpiration in desert plants. He spends three months at the Desert Botanical Laboratory for this purpose.—*Science*.

IT IS ANNOUNCED by *Science* that Professor DOUGLAS H. CAMPBELL will spend next year in an extensive trip through Europe, Africa, and Asia. He will visit the regions about Victoria and Zambesi Falls; the botanical gardens at Paradeniya and Buitenzorg; and will then return by way of the Philippine Islands.